GENETICS

A periodical record of investigations bearing on heredity and variation

Founded in 1916 by GEORGE H. SHULL

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VOLUME 44 SEPTEMBER, 1959 NO. 5, PART 2

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1. GENETICS will be changed from a bimonthly to a monthly journal.

2. The annual volume of the journal will include a minimum of 1,000 pages. The Editors expect to include enough additional pages to print all acceptable manuscripts if funds are available.

3. The subscription rate will be $12.00 for a complete volume (January-December). Foreign postage will be $1.00 extra. Single copies will cost $1.50.

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a) Manuscripts must conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of Literature Cited, and inclusion of a Summary.

b) A gene symbol should be defined or identified with the mutant character the first time the symbol is used in a manuscript.

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STUDIES IN QUANTITATIVE INHERITANCE. XII. CELL SIZE AND NUMBER IN RELATION TO GENETIC AND ENVIRONMENTAL VARIATION OF BODY SIZE IN DROSOPHILA

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INDIVIDUALS may differ in body size for many distinct reasons, both environmental and genetic, and it is of some interest to know how far different causes of variation and also different kinds of genetic behavior are associated with characteristic differences in development. Mere numerical record of genetic variation of body size can identify different types of genetic behavior but lumps together different effects which must be recognized and studied if a fuller understanding is to be achieved. Previous publications in this series have been concerned with various aspects of the genetic variation of body size in *Drosophila melanogaster* as revealed by selection, inbreeding, chromosome interchange between lines and other tests. It is now necessary to examine such situations from a rather different viewpoint and see whether the combination of genetic and physiological evidence can take the analysis to a deeper level.

Ideally the size of an animal or an organ can be described in terms of the size and number of its cells and it would be valuable to know how the cell size and number relations change in different genetic and environmental situations. Such information should, at the same time, throw light on the regulation of body size and of genetic differences in this capacity.

It so happens that an insect like Drosophila is quite well suited for such a study. DOBZHANSKY (1929) first pointed out that changes in cell size and number might be estimated from the cell density in the wing membrane which consists of a double layer of cells, each bearing a tiny bristle. The cell outlines can be seen shortly after eclosion but later become obliterated. The number of bristles which occur in a given area of upper or lower surface of the wing provides a measure of the surface area of a cell or cell size. Although gradients in bristle density occur, the cells are regularly arranged and counts in different regions of the wing are quite highly correlated. It is well known that wing and body size vary together when nutrition is altered and many observations (ROBERTSON and REEVE 1952; REEVE and ROBERTSON 1953) have demonstrated a high genetic correlation as well and so differences in wing area generally afford a reliable indication of comparable differences in body size. Hence records of wing area and cell density provide the basic data for this study in which the variation of size and cellular constitution of a single organ, which is highly correlated with body size, is used as an indication of genetic or environmental differences which influence body size generally.

With respect to earlier work, DOBZHANSKY (1929) demonstrated a striking correlation between wing cell size and estimates of total chromosome volume—disregarding the Y—among the progeny of a cross between diploid and triploid flies which produces a variety of numerically unbalanced chromosome combinations. ALPATOV (1930) studied the changes caused by rearing flies at different temperatures and levels of crowding and reported a general tendency for crowding to be associated with changes in both cell size and number while temperature affected cell size. ZARAPKIN (1934) compared cell size in large and small selected strains of *Drosophila funebris* and concluded that changes in cell number were entirely responsible for the observed differences in wing size. More recently ROBERTSON and REEVE (1952) reported that differences in wing area between a large and small strain of *Drosophila melanogaster*—also selected for wing size—could be attributed to changes in cell size. However, in the small strain wing size was disproportionately reduced—relative to thorax length—by sex-linked genes and this may account for the apparent discrepancy with ZARAPKIN's data, especially since DOBZHANSKY (1929) noted that the sex-linked miniature which reduces wing size does so *via* cell size. BREHME (1941), in a study of the development of different Minute mutants which grow more slowly than normal, found the wing cells reduced in size and suggested that reduction of body size could be attributed to change in cell size. BARIGOZZI (1951) reported differences in cell size when the Y chromosome from different stocks was placed against a standard genetic background. These various observations underlined the need for a more systematic study which lead to the collection of rather extensive data, which, for reasons of space, cannot be accommodated in a single paper. Hence the present paper deals with the effects of environmental variation, estimates of genetic variation in wild populations, and the results of selection for large and small cell size. The next paper in this series will be concerned with the changes in cell size and number which are associated with selection for differences in body size, crosses between lines and other special genetic situations.

MATERIAL AND METHODS

General: In view of the correlation between counts in different regions of the wing and the labor involved in scoring many individuals, cell size has been estimated from a single region, midway between veins III and IV and a little distal to the major cross vein. The area examined represents approximately one percent of the total wing area at 25°C. Wings are mounted on slides to allow projection of an enlarged image on a ground glass screen. To facilitate counting and provide a check on the homogeneity of the bristle distribution the screen was provided with a ruled grid, divided into a number of equal areas. Wing area is determined from a magnified outline with the aid of a planimeter. The measure of cell size refers to the wing. How far the cell size and number relations in the wing hold also for the rest of the exoskeleton which has a similar embryological origin and growth during the larval period, will be considered later; naturally there is no reason to expect parallel behavior in different kinds of tissue like gut or gonad.

General details of culture on the maize meal molasses medium, measurement of live flies and so on have been described in the first number of this series (ROBERTSON and REEVE 1952). Comparison of the average dimensions of different series is based generally on records for 40–50 females drawn equally from four or five replicated cultures.

The effect of inadequate nutrition on the relations between cell size and number in individuals of different genotype have been studied by culturing larvae on a chemically defined, aseptic medium developed by SANG (1956). Suboptimal levels of specific, essential nutrients reduce adult size, which can be influenced by many different kinds of nutritional imbalance. It is thus possible to discover how individuals of the same genotype respond to different treatments which produce an equivalent reduction in size and also how far genetically diverse individuals differ in response to the same treatment.

Statistical analysis: The analysis of the data presents a few problems. Since the area of the wing may be regarded as the product of cell size and number, a log transformation is required to convert these variables to an additive scale. Accordingly all records of wing area and cell area were converted to natural logarithms before analysis. The estimate of "cell size" is simply the area in which bristles are counted divided by their number. In the tables which follow, mean wing area and cell area or cell size are expressed in terms of log squared hundredths of a millimeter. The region of the wing scored was chosen because it is fairly central and the bristles are regularly distributed. SCHATZ (1951) working with various wing mutants described striking changes in the pattern of bristle density. However, many tests indicate that major heterogeneity of bristle density is absent in the material studied here and that average cell area in the region studied is quite adequate for comparison with changes in wing size. Since the counting grid was divided into six equal areas, it was possible to check the uniformity of the bristle density. The variation of a unit area may be regarded as made up of a component (c) common to all such units of the total area studied and an error component (i) to give $x = c + i$ as the variance of a unit; the approach is similar to that used by REEVE and ROBERTSON (1954) in the study of the number of bristles on successive abdominal sternites. The variance of the total area of n units is given by $V\,\Sigma\,(x) = n^2\,\sigma_c^2 + n\,\sigma_i^2$ while the sum of the variances of the units areas is represented by $\Sigma\,(V\,x) = n\,\sigma_c^2 + n\,\sigma_i^2$. From these relations we can deduce

$$\sigma_c^2 = \frac{V\,(\Sigma\,x) - \Sigma\,(V\,x)}{n(n\text{-}1)}\,;\ \sigma_i^2 = \frac{n\,\Sigma(V\,x) - V\,(\Sigma\,x)}{n(n\text{-}1)}$$

If the wing cells and their bristles are entirely homogeneous, σ_i^2 will represent the random variation arising from the application of a fixed outline to a discontinuous distribution; departure from a uniform distribution will inflate σ_i^2. σ_c^2 represents the best estimate of the variation of cell size after the error variation has been allowed for. When cell size is derived from the total bristle count, the correlation between wing and cell size must be corrected to allow for the inclusion of this error variance.

In practice it turns out that, although minor heterogeneity is present, there is

considerable consistency among a variety of genotypes in the estimates of σ_i^2. Evidence is available from a series of inbred lines of diverse origin, of crosses between them and also from various wild stocks and selected strains. Table 1 shows the average error variance of a total count of six units ($\sigma_i^2/6$), weighted by the degrees of freedom in each sample and from this we conclude that the average value of 0.000,782, derived from the pooled samples is a suitable estimate of the error variance of total area. When the accumulation of data had demonstrated the consistency of σ_i^2 this was turned to advantage by using the average value and the variance of the total count to calculate σ_c^2 indirectly, thereby lightening the effort of computation. In the data presented below estimates of the variance of cell size refer to σ_c^2 and regressions of wing on cell size allow for this. Errors of counting and positioning of the grid make a negligible contribution to the variance of total count; this was checked by repeat counts on a number of wings.

It is worth noting in Table 1 that the estimates of σ_i^2 from inbred line crosses and wild stocks are about the same, i.e., there is no evidence of any change in the degree of heterogeneity of bristle pattern as a result of inbreeding.

Log cell number is estimated from the difference between log wing area and log cell area. For graphical representation the regression of log wing area on log cell area provides a convenient guide to changes in cell size or number which accompany a given change in wing size. Thus if the cell number is constant we expect a regression slope of unity. Values intermediate between zero and unity suggest an inverse relation between the two variables while values greater than one imply that both are changing in the same sense. With these considerations in mind we are now in a position to consider what happens to cell size and number in different situations.

EXPERIMENTAL RESULTS

Environmental influences

Temperature: It is well known that larvae grown at lower temperatures produce bigger flies (ALPATOV 1930) and also that the relative size of different parts of the body vary according to temperature (IMAI 1934). Tests on wild stocks have shown that between 30°C and 18°C wing area changes two to two and a half times as much as the square of thorax length. This effect of temperature is the most notable exception to the regularity with which wing and thorax dimensions show similar proportional change. Since ALPATOV's (1930) tests, carried out on a

TABLE 1

Error variance of cell counts per total area in different genotypes

Genotype	Degrees of freedom	Error variance
Inbred lines	356	0.000,773
Crosses between lines	255	0.000,769
Wild populations	814	0.000,789
Weighted average	...	0.000,782

single strain, suggested that variation of wing size due to temperature is a function of cell size, it is convenient to begin by seeing how far this is generally true. For this purpose, pairs of lines, long inbred by brother-sister mating, together with their F_1 crosses were reared at three temperatures: 18°, 25°, and 30°C. The crosses were made between the lines N_2 and C_6, N_2 and E_4, R_2 and C_9, C_9 and C_{10}; the symbols N, R, E and C refer to the origin of the lines from the Nettlebed, Renfrew, Crianlarich and Edinburgh wild stocks. Five cultures of eggs were generally set up for each line and cross and 6–8 females were scored from each culture. Figure 1 a-d shows the mean values of wing area and cell size; the following features are to be noted:

(i) Taking the average of the crosses as representative of the behavior of non-inbred wild flies, it appears that a change of temperature from 30° to 18°C increases wing area by some 45 percent. A glance at the different graphs in Figure 1 shows that this change can be attributed almost entirely to a change in cell size and that cell number remains comparatively constant. The line with a slope of one is drawn through the average wing area and cell size of the crossbred flies reared at the three temperatures and it is clear that the points for the crossbred flies fall closely about this line in every case.

(ii) Although the inbred lines follow the same general rule they show less consistency in their response to temperature, especially when cultured at 30°C. Wing area is smaller than anticipated from the relations observed at 18° and 25° C, especially in lines C_6, C_{10} and R_2. Since 30° C is near the upper limit of growth and survival it is to be expected that inbred lines will be more adversely affected than the crosses and the discrepancy in wing size is presumably a reflection of this. The disproportionate reduction of wing area at the higher temperature is reflected mainly in a lower cell number. We can estimate log cell number from the difference between average log wing and cell area and such estimates for the various series at 30°C, expressed as deviations from estimated cell number at 18°C, are set out in Table 2. The inbreds show a consistently negative deviation with an average of -0.092 compared with -0.012 for the crosses.

(iii) Inspection of the graphs shows that the relations between cell size and number for a given wing size are not necessarily the same; the special case of flies cultured at 30°C are excluded from this consideration. The point is well made by comparing wing area and cell size of lines N_2 and E_4 at 25°C in Figure 1b. Although the wing area in the lines is about the same, the cell sizes work out at 0.703 and 0.583 log units—a highly significant difference. A similar situation is seen in Figure 1c with respect to the dimensions of R_2 and C_9 at both 18°C and 25°C. This raises the general problem of genetic variation in the cell size and number relations, independent of wing size—a topic to be considered later.

(iv) Although all genotypes, apart from the special changes at 30°C, tend to follow the same pattern of response to temperature change nevertheless the extent of the shift up and down the slope of unity is not constant, i.e., the difference in wing area between the contrasted genotypes is not the same for a given temperature difference. Thus in Figure 1a, the F_1 of the cross between N_2 and C_6 signifi-

TABLE 2

Difference between estimated log cell number at 30°C and 18°C

Parents	Deviations	
	Inbred lines	Crosses
C_6	−0.081	−0.027
N_2	−0.010	
E_4	−0.166	−0.018
N_2	−0.022	
R_2	−0.055	−0.013
C_9	−0.070	
C_{10}	−0.231	−0.025
C_6	−0.101	
Average	−0.092	−0.012

cantly exceeds the larger line N_2 at 18°C, but at 25°C it is slightly smaller. In Figure 1b N_2 is bigger than E_4 at 18°C but is smaller at 30°C. In Figure 1c the F_1 between R_2 and C_9 is much larger than either parent at 18°C whereas at 25°C all genotypes have about the same size. Finally in Figure 1d, at 25°C the cross exceeds both parent lines which are about the same size, while at 18°C C_{10} and the F_1 are very similar and considerably exceed C_6. As a result of this genotype environment interaction the size of the F_1 in relation to the size of the parents may differ according to the temperature. Hence dominance deviations estimated from the comparison of the sizes of parents F_1, F_2 and backcrosses would be quite different according to the temperature during development.

(v) In recent years there has been discussion of two aspects of the response to environmental variation of individuals which differ genetically in such a way that we may reasonably regard one genotype as conferring greater fitness than another, as when we compare inbred lines and crosses. On the one hand, there is plenty of evidence that the latter achieve greater stability of phenotype in that certain characters like size and fertility are less variable. But there is also the possibility that the fitter types have greater capacity to respond adaptively to different conditions so that, in certain respects they will be more variable (SCHMALHAUSEN 1949; THODAY 1955). Such effects are familiar in plants but apparently rather difficult to demonstrate with assurance in animals; a good example is described by SMITH and SMITH (1954).

In the present context the evidence rests on whether the crosses show greater or less response to a given temperature difference than their inbred parents. For this purpose we can compare performance at 25°C and 18°C; tests at 30°C are excluded for the reasons given above. The tendency for wing size to be disproportionately reduced in inbred lines at 30°C could be regarded as evidence of the greater stability of crosses.

Treating the eight sets of data from inbred lines and four from crosses, as two groups for comparison, the crosses do indeed show a greater difference in wing area and cell size between 18° and 25°C than the inbreds. Table 3 shows the average difference in wing and cell size at the two temperatures. For wing area, the average difference works out at 0.218 and 0.249 units for inbred and crosses respectively, while for cell size the corresponding values are 0.193 and 0.268, with a difference of 0.075, which is significant at the 0.02 level of probability.

Although the difference for wing size is not statistically significant since it goes in the same direction as cell size, the effect is probably real. Thus these comparisons of performance at 25°C and 30°C illustrate the greater ability of cross-

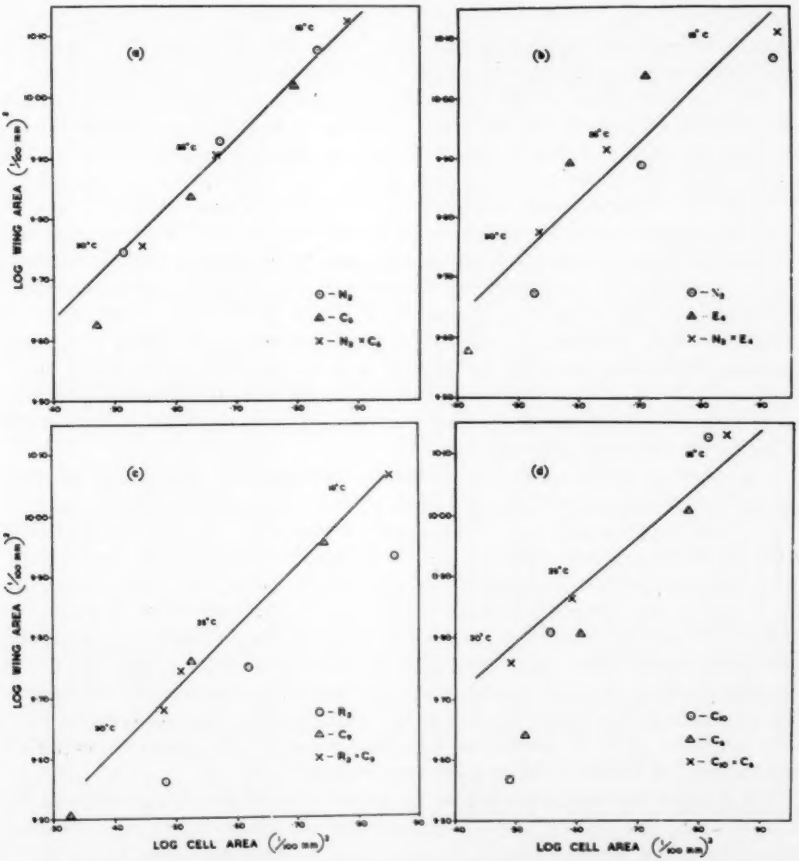

FIGURE 1.—Cell and wing areas in inbred lines and crosses at different temperatures.

TABLE 3

Average difference in wing and cell area between inbred and crossbred flies reared at 18°C and 25°C in log units

	Wing area	Cell area
Inbred lines	0.218 ± 0.018	0.193 ± 0.016
Crosses	0.249 ± 0.025	0.268 ± 0.022
Difference	0.031	0.075
P	>0.05	<0.02 > 0.01

breds to withstand more extreme conditions, while performance at 25°C and 18°C points to their greater responsiveness to this particular environmental change.

(vi) The inbred line N_2 was used in the cross to C_6 and also E_4. The tests were separated by an interval of five weeks and there is no reason to suppose that N_2 changed genetically in that period. However, at corresponding temperatures, the wing area in the second test is less than in the first; this is shown in Figure 2. At 30°C and 25°C the differences between the estimates of both cell and wing area in the two experiments are about the same, although at 18°C wing area is relatively a little bigger in the later test. Environmental differences between the tests may be due either to temperature or nutrition. It is improbable that temperature differences are the cause. It is obvious from Figure 2 that most of the observed difference in wing size must be attributed to cell number, so there is an interesting contrast between the effects of temperature, which influence cell size and of nutrition, which here influences cell number. This suggests that the next logical step is to examine the effects of variable nutrition on cell size and number; such experiments are dealt with next.

The effects of variable nutrition: Nutritional effects have been studied by rearing larvae on chemically defined aseptic media which contain all the essential nutrients in excess except for certain specific deficiencies; the most favorable diet is provided by medium C of SANG (1956). Suboptimal levels of any essential nutrient reduce body size. This procedure is preferable to the reduction of body size by crowding on live media since it enables us to compare different genotypes under similar conditions of nutritional imbalance, while the homogeneous, aseptic medium reduces within culture variance to a low level. The compounds which have been reduced in concentration comprise casein, ribonucleic acid and choline. Three unrelated wild populations, namely Pacific, Gabarros and Kaduna have been reared on media deficient in one or other respect; in the Kaduna population only casein and choline deficiency has been studied.

The data are summarized in Figure 3a, b, c in terms of the average cell number and cell area recorded for the different treatments. Since we are not concerned with differences between strains in nutritional requirements, this aspect of the data will not be considered here. The first point to note is the general tendency

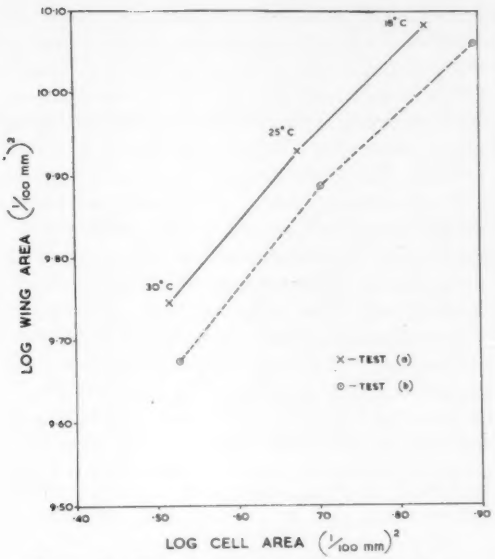

FIGURE 2.—Dimensions of line N_2 in different tests.

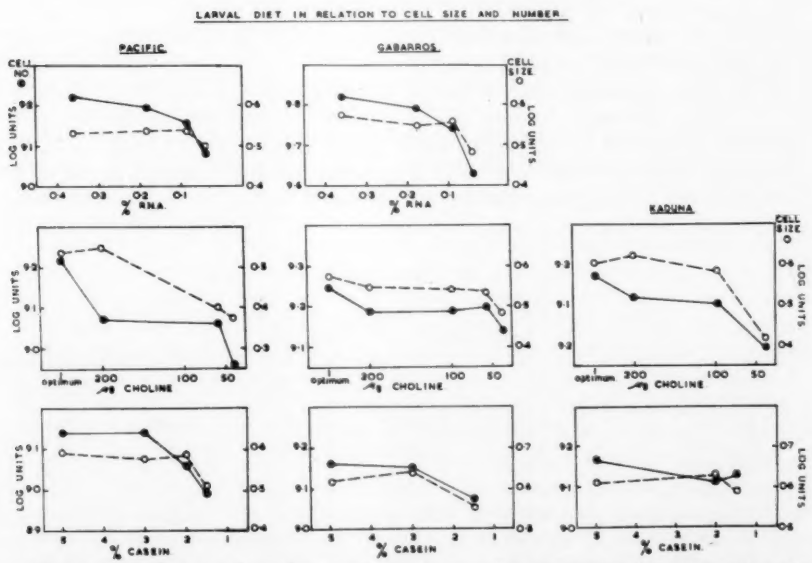

FIGURE 3.—Cell size and number in wild strains reared on chemically defined aseptic media which differ in concentration of particular essential nutrients.

for nutritional imbalance to affect cell number more readily than cell area. This is especially evident in the Pacific and Kaduna populations in which a considerable decline in wing size may leave cell size unchanged. The Gabarros strain apparently differs from the other two in that cell size is more readily affected. When conditions become too adverse, both cell size and cell number are reduced. A particularly interesting situation is presented by the effect of choline deficiency in the Pacific population. The initial decline in wing size due to culturing on the synthetic medium supplied with 200 μg of choline instead of the usual live yeast or "optimum" medium involves a decline in cell number alone. But the difference in wing area between flies reared on media with 200 and 60 μg choline is apparently almost entirely due to a reduction of cell area whereas further reduction of choline concentration is accompanied by decline in both cell size and cell number.

The most important feature of these results is the general tendency for unbalanced diets which lengthen the period of development and reduce body size to be associated with a decline in cell number rather than cell size. Other experiments, with inbred lines and crosses, not dealt with here, support this general conclusion. It will be recalled that the differences of wing size between the two tests with line N_2 were attributed to differences of cell number. So this behavior appears to be quite typical unless starvation is too acute.

Genetic variation

Comparison of wild populations: It is convenient to consider first the dimensions of different wild populations. Table 4 summarizes the data for seven populations derived from widely separated localities and kept in the laboratory for varying periods of time, generally several years, but only a few weeks in the case of Pacific. The populations are tabulated in order of diminishing size. There are well defined differences in body size between the populations; the extremes differ by some 11 percent in terms of squared thorax length. Average wing and thorax size are highly correlated ($r=0.72$). The end column of Table 4 lists the difference between log wing area and log squared thorax length, which provides a measure of relative wing size. Five of the strains are similar in this respect, while one,

TABLE 4

Average wing area, cell area, cell number and squared thorax length in wild populations; in log units

Populations	Thorax	Wing area	Cell area	Cell number	Wing-thorax difference
Renfrew	9.420	9.837	0.537	9.300	0.417
Gabarros	9.381	9.907	0.599	9.308	0.526
Sao Paulo	9.380	9.848	0.552	9.296	0.468
Pacific	9.353	9.806	0.559	9.247	0.453
Crianlarich	9.348	9.799	0.549	9.250	0.451
Ischia	9.329	9.783	0.580	9.203	0.454
Edinburgh	9.312	9.765	0.577	9.188	0.453
Standard error	0.008	0.006	0.006	0.006	....

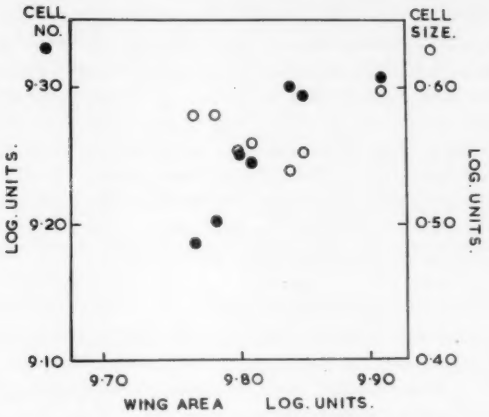

FIGURE 4.—Cell size and number in wild populations.

Renfrew, has relatively small wings and another, Gabarros, relatively large wings, and these differences are associated with larger and smaller cell area. This may indicate that major variation in relative wing size is associated with change in cell area rather than number. The data are represented graphically in Figure 4 by plotting average log cell area and log cell number against log wing area. This brings out clearly the importance of cell number; the regression of wing area on cell number works out at 0.90 ± 0.19. One population, Gabarros, deviates more than the others from the regression line and this is obviously associated with its larger relative wing size. Average cell area is comparatively constant. There is some evidence of an inverse relation between average cell area and cell number but this is quite minor compared with the major variation of cell number. The significance of this relative constancy of cell area in populations which differ in body size will be discussed later.

The variation of cell size. General: The genetic contribution to total phenotypic variance can be roughly estimated by comparing the variance among individuals of a wild population with the variance of appropriate genetically uniform individuals. General experience suggests that crosses between inbred lines are suitable for such comparisons since their general reaction to environmental variation is much the same as that of wild individuals. The variance of any character for such genetically uniform flies can be taken as a measure of environmental effects. Records of wing and cell size from several different wild populations and also from crosses between various inbred lines are available from experiments carried out at different times. Since the estimates of variance and covariance within each of the two categories present a considerable hemogeneity such within-culture estimates have been pooled to provide a reliable picture of the average variance under optimal conditions. The estimates are set out in Table 5 in which the components have been multiplied by 10^6 and rounded to the nearest ten to make com-

TABLE 5

Genetic and environmental components of variance and covariance (log units: $\times 10^6$)

Genotype	Direct estimates						Indirect estimates				
	d.f.	σ^2_S	σ^2_C	cov_{SC}	b_{SC}	r	σ^2_N	cov_{SN}	b_{SN}	cov_{NC}	b_{NC}
Variable	271	2190	1640	330	0.20	0.18	3160	1850	0.59	—1300	—0.80
Uniform	89	710	510	0.00	0.00	0.00	1220	710	0.58	— 510	—1.00
"Genetic" effects	...	1480	1130	330	0.30	0.26	1940	1140	0.59	— 790	—0.71
Percentage of total	...	67	69	100	...	...	61	62	...	....	...

S, *C* and *N* refer to wing area, cell area and cell number.

parison easier. Estimates of genetic effects are simply the difference between corresponding components for wild and genetically uniform flies, i.e. genetic and environmental effects are assumed to combine additively on a log scale, which, although unlikely to be strictly true, is a good enough approximation when environmental conditions are favorable.

As expected from previous work, there is substantial genetic variation of wing size, accounting for a little under 70 percent of the total variance. Genetic variation of cell size works out at about the same level. A regular feature of wild populations is the low, but consistently positive regression of wing area on cell area which works out at 0.20. This correlation is evidently entirely genetic in origin since the covariance terms in the crosses is zero i.e. the nutritional variation within cultures leaves general cell size unchanged, as might be expected from the evidence presented earlier in the section dealing with nutrition. The genetic regression of wing on cell area rises to 0.3. This correlation could arise from an association between wing cell size and either general body size or relative wing size, or both could contribute to the variance. However, general experience of the high level of genetic correlation between wing and thorax size in wild populations and also the finding that log squared thorax length and log cell area are also correlated to a similar degree in the wild populations examined (r=0.21 for 130 degrees of freedom) suggest that the observed correlation between wing and cell area reflects variation of general body size. Thus although most of the variation of wing and hence body size is associated with variation of cell number, nevertheless an appreciable fraction is associated with variation of cell size.

It may be further inferred that there is a high negative correlation between cell size and number as the following considerations show. Since the area of the wing can be regarded as the product of average cell size (C) and number, (N), or the sum of the log values, the variance of wing size (S) can be expressed as

$$\sigma^2_S = \sigma^2_C + \sigma^2_N + 2\ cov\ CN$$

from which by substitution and rearrangement it follows that

$$\sigma^2_N = \sigma^2_S + \sigma^2_C - 2\ cov\ SC$$

$$cov\ SN = \sigma^2_S - cov\ SC$$

$$cov\ CN = cov\ SC - \sigma^2_C$$

Such indirect estimates of the variance of cell number together with the covari-

ance between wing size and cell number and also cell size and number are shown on the right hand side of Table 5. They draw attention to the high negative correlation between cell size and cell number evident in both environmental ($r = -1.0$) and also genetic effects ($r = -0.54$). Such indirect estimates must naturally be treated with a certain reserve, since error variance in the estimates of average wing cell size will contribute to apparent negative covariance between cell size and number. It is most improbable that the observed negative covariance is merely due to this fact as the later results show. The negative covariance of cell size and number suggests that genetically different individuals of the same wing size may differ in cell size and number. Such variation tends to obscure underlying relationship associated with change of wing size and special tests are needed to clarify the situation.

The effects of selection: Since there is plenty of genetic variation influencing cell size in wild populations, further information about its properties was gotten by selection for large and small cell size in three different wild populations. Selection was carried out for 2, 3, and 9 generations in the Pacific, Pobla de Lillet and Gabarros populations respectively. The procedure was as follows: from each of four cultures, 15 pairs of newly emerged flies were drawn at random, the left wing cut off carefully, mounted and the extreme four pairs with the highest or lowest cell density were selected from each culture and combined with similarly selected individuals to form the parents of successive generations. Thus each generation 16 pairs of flies were chosen from a total of 60 pairs scored. The removal of a wing had no obvious adverse effect on the flies, which mated with alacrity when the selected parents were put together. Throughout these tests there was no evidence of any change in survival, as measured by the proportion of eggs cultured which became adults. Samples from the unselected population were set up sufficiently often to compare the effects of selection in either direction. The results with the different populations are described in turn.

(a) *The Pobla de Lillet population.* In this test selection ran for three generations. Table 6 shows the average cell size in the successive generations, sexes averaged, and also the differences between the selected strains.

Cell size in different generations cannot be compared directly since controls were not raised and it is known that temperature fluctuated about 3°C during this

TABLE 6

The effects of selecting for large and small cell size in the Pobla de Lillet population

Mean cell size of the selected strains: sexes averaged, log units			
Generation	Large	Small	Difference
1	0.550	0.509	0.041
2	0.550	0.489	0.061
3	0.559	0.489	0.070
Cumulated selection differential			0.284
Estimated heritability			0.247
Standard deviation in unselected population			0.050

selection experiment. This does not affect the comparisons between the selected strains reared together. Evidently cell size responds quickly to selection and after three generations the strains differed by some seven percent which corresponds to about one and a half times the standard deviation of cell size in the unselected population. Heritability can be estimated from the ratio of final difference between the selected strains to the cumulated selection differential i.e., the sum of the average selection differentials per generation, estimated as the deviation of the selected parents from their culture means. With this criterion, heritability works out at about 25 percent.

It might be thought that the selection response is due to a change in the pattern of cell density rather than a change in average wing cell size. To check this counts were carried out on 24 flies of each strain in three additional regions of the wing where the density differs somewhat. These regions were located in the distal region of the marginal cell (II), between veins II and III about three quarters of the way along the veins (III) and roughly in the middle of the third posterior cell (IV); the usual region for counting is labelled 1. The ratio of cell density in the small to that in the large cell strains worked out as follows for the different regions:

I	II	III	IV
1.076	1.062	1.070	1.053

Thus, although minor shifts in pattern may have occurred, these are certainly trivial compared with the average change in cell size throughout the wing.

From the last generation of selection and also the unselected population eggs were collected and set up in five cultures; virgin flies were also crossed reciprocally. Since the direction of the cross made no difference the data have been combined; ten females were scored from each culture; the relevant comparisons are shown in Table 7.

Since the difference between the selected strains in this test and at the end of selection is virtually identical—0.076 versus 0.070 units—the generation of relaxed selection was apparently without effect. Comparing the selected strains with controls, the large moved further away from the initial level than the small cell strain i.e. 0.045 compared with 0.031 units. Wing size has declined in both selected strains, especially the small cell strain. The decline in wing size of the large cell strain is unexpected in view of the positive genetic correlation found

TABLE 7

Comparison of selected strains and crosses with the unselected population: females only

Genotype	Deviation from unselected (log units)		
	Cell area	Cell number	Wing area
Large cell strain	0.045**	—0.074	—0.029*
Small cell strain	—0.031**	—0.014	—0.045*
Cross	—0.006	—0.013	—0.019

* Indicates significance of the 0.05 level of probability.
** Indicates significance of the 0.01 level of probability.

among individuals of the unselected stock. The F_1 flies do not differ appreciably from the average values found in the unselected stock, suggesting that the re-establishment of more normal cell size and number relations and wing size are interrelated.

To summarize, this experiment shows that (a) there is an inverse relation between cell size and number, with respect to selection for large cell size since striking increase in cell area is accompanied by decline in cell number. (b) It is easier to change the cell size and number relations in favor of larger cells and fewer of them than *vice versa*. (c) The selection for small cell size which leads to a decline in wing size may be relevant to the positive correlation between wing and cell size found among individuals of the unselected population (Table 5).

(b) *The Pacific population:* Two generations of selection for large and cell size were carried out in this population; the results are summarized in Table 8. The response was quite striking, amounting to a difference of some seven percent between the selected strains—equivalent to about one and a half times the standard deviation. Again selection was more effective upward than downward leading to a deviation from the control of 0.043 log units as compared with 0.028 and this is reflected in the estimates of heritability which work out at 0.58 and 0.47 respectively; these values are higher than in the last experiment. Apparently selection has affected only the cell size and number relations apparently independently of wing size, since this has remained constant.

(c) *The Gabarros population:* The selection response is summarized in Figure 5, a-c. The top graphs (5a) which show the deviation from the controls in successive generations, indicates that selection has produced a striking effect in either direction. By generation 9 the cell area in one strain is about 20 percent greater than in the other. The nature of the response is rather different in the two directions; in the large cell strain there was a fairly steady response for five generations, after which response apparently ceased. In the other strain, the initial response was slower and then speeded up and was apparently still continuing when the experiment was discontinued at generation 9. If records of cell size were the only data available, i.e., if cell size were being treated as a "character", the nature of the selection response might invite comparison with one or other of the characteristic types of response found when other characters are selected for. However, it is easy to show that inferences based on such comparisons would be of little value, while heritability estimates derived from the apparent response

TABLE 8

Response to selection for cell size in the Pacific population

Selected strain	Deviation from unselected				
	Cell area	Cell no.	Wing area	Selection differential	Heritability
Large cell	0.043**	—0.048	—0.005	0.074	0.58
Small cell	—0.028**	0.029	0.001	0.064	0.44
Standard deviation of cell size = 0.045					

** Indicates significance of the 0.01 level of probability.

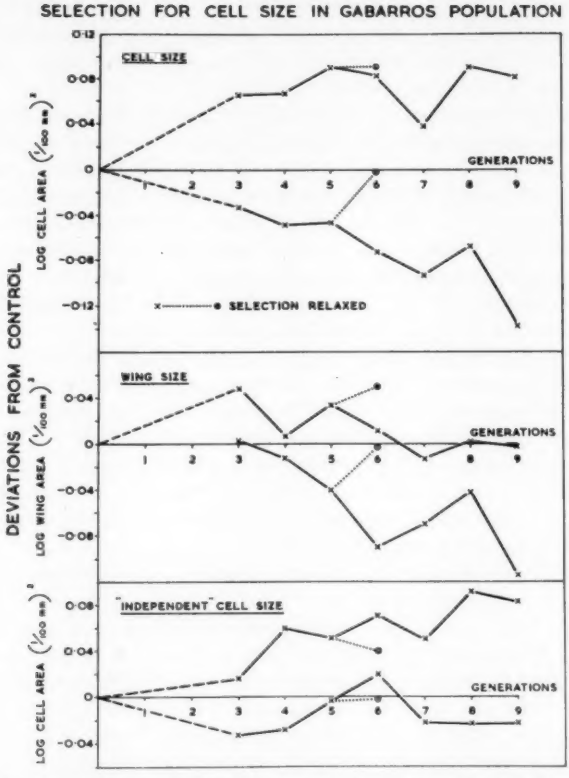

FIGURE 5.—In the bottom graph the points are calculated by subtracting the deviation from control for wing area from the corresponding deviation for cell area. This gives an indication of shift in the inverse relations between cell size and number—hence the term "independent" cell size.

would be very misleading. Figure 5b shows what happened to wing size during selection. In the large cell strain, wing size increased during the first three generations and then declined irregularly to fluctuate about the control level. Thus the initial response to selection apparently involved effects correlated with body size rather than merely changes in the cell size and number relations within the wing, but in view of the data presented later, the correlation may be due to linkage.

In the small cell strain the picture is reversed. The first three generations of selection leave wing size unchanged, but from generation 4 onwards there is a drastic decline in wing size, so that by the end of the experiment, the latter has fallen about ten percent below the original level. The later response to selection

is due to genetic changes which affect general cell size, rather than changes in the cell size and number relations within the wing.

Since average wing and cell size may be expressed as deviations from the control level, the changes in cell area which are apparently independent of the changes in wing area and which involve inverse changes in cell number can be estimated by subtracting the deviation for wing from that for cell size; this is illustrated in Figure 5c. With this procedure, there is a marked asymmetry of response; in the large cell strain the cell area is increased about eight percent while in the other strain the decline is only about two percent. This recalls the similar trend in the Pobla de Lillet selection, suggesting that it is a general rule for wild populations, that it is easier to shift the cell size and number relations, within the wing, in favor of larger cells and fewer of them. The substantial reduction of wing size effected by selection for cell size alone is reassuring evidence that the measure of cell size is a perfectly adequate indicator of average cell size in the wing.

At generations 3, 4, 7 and 9, thorax length was also recorded to see whether the changes in wing size reflect changes in general body size. The comparisons are set out in Table 9, in terms of deviations from the average values for the unselected population. The dimensions of thorax are expressed as the log of the square of the length, to make the deviations more comparable with wing area; only females were studied. Since the data are in natural logs and the deviations are not too great, the values in Table 9 can be converted into approximate percentage differences by multiplying by 100.

Allowing for the likelihood that equivalent changes in body size are not expressed by precisely equivalent changes in the log of wing area and the square of thorax length, and also for minor fluctuations of temperature which, as noted earlier, affect wing more than they affect thorax size, the trend of the deviations in both selected strains leaves little doubt that changes in wing area which accompany changes in cell area reflect changes in general body size. At generation 5 eggs were set up from the selected and unselected strains in five replicates at 18°C, 25°C and 30°C; ten females per culture were scored. The effects of temperature will be considered later; at present we are concerned only with the dimensions of the strains reared at 25°C after a single generation of relaxed selection as shown

TABLE 9

Wing and thorax size in the Gabarros selection experiment

	Deviations from controls: in log units			
	Large cell strain		Small cell strain	
Generation	Wing	Thorax	Wing	Thorax
3	0.049	0.044	0.001	0.017
4	0.006	0.089	−0.012	0.070
5	0.033	−0.013	−0.071	−0.019
6	0.001	0.004	−0.041	−0.037
7	−0.002	−0.020	−0.115	−0.086

in Figure 5. Since the flies in this test were not raised along with the flies of generation 6, the two groups can be compared by reference to their deviations from the control level.

In the large cell strain, cell size was apparently unchanged after a generation without selection, but wing area increased. In the small cell strain wing and cell size reverted to the control level. There is complete correlation between the wing and cell size in the behavior of the small cell strain, whereas in the large cell strain the increase in wing size involves a shift in the cell size and number relations.

Since the genetic variation of cell size is made up of qualitatively different effects which contribute very unequally to the response in either direction, estimates of heritability derived from the ratio of response to cumulated selection differential might conceivably have some empirical value if merely variation in wing cell size alone were considered, but they would have scant biological significance. Perhaps a slightly more meaningful picture can be arrived at by confining such estimates to periods during which there is either little or no change in wing size or to periods when there is a high correlation between wing and cell size.

In the former situation, variation in the inverse relations between cell size and number predominates, whereas in the latter the physiological nature of the variation differs. Thus by reference to Figure 5, one might estimate heritability for the independent effects in generations 7 through 9 in the large cell strain, and for the first three generations in the other strain. Also if one assumes that selection for cell is essentially selection for wing size after this period, and is further prepared to relate the selection differential for cell to the response in wing size, a heritability estimate for the latter can be computed. Such estimates are admittedly very rough approximations; but they are summarized in Table 10.

At the end of the selection experiment the strain selected for small cell size was crossed to the unselected population, and the F_1 reared along with the two parent strains The results shown in Table 11 are expressed as deviations from the average values recorded for the small cell strain. The last column refers to relative wing size i.e. log wing area minus log squared thorax length.

As noted earlier, the small cell strain is appreciably smaller than the unselected population; squared thorax length is some eight percent smaller. When crossed to the unselected stock, average thorax size of the F_1 coincides almost exactly with that of the unselected stock, so the difference in general body size created by selection for small cell sizes behaves as entirely recessive. Wing and cell area also increase in the F_1 beyond the level of the small cell strain but at first sight there

TABLE 10

Heritability estimates for wing and cell size in the Gabarros for selection

Strain	Dimensions	Generation interval	Response log units	Selection differential	Heritability
Large cell	Cell size	6–8	0.028	0.129	0.22
Small cell	Cell size	1–3	0.024	0.155	0.15
Small cell	Wing size	4–9	0.115	0.296	0.39

TABLE 11

The effects of crossing the small cell Gabarros strain to the unselected population: in log units

	Deviations from the small cell strain				
	Squared thorax length	Cell area	Cell no.	Wing area	Wing—thorax
Unselected	0.077	0.167	—0.044	0.123	0.046
Cross	0.078	0.116	—0.045	0.070	—0.008
Difference	0.001	0.051	0.011	0.053	0.054

is a discrepancy in that wing size, unlike thorax size, is not increased up to the control level. This can be explained by referring to the differences in relative wing size listed in the end column of Table 11. The cross and the selected strain have the same relative wing size which is less than that of the unselected stock. Furthermore, this discrepancy between the unselected flies and the F_1 can be associated with the relatively larger cell area of the former, since cell number works out at the same value for the F_1 and the unselected stock. Thus, in this cross relatively larger wing size, associated with correspondingly larger wing cells behaves as recessive. Apart from this difference in wing-thorax ratio, there has been a complete restoration of body size and cell number in the wing.

Thus selection for small cell size has involved a syndrome of effects which include general body size as well as the cell size and number relations in the wing. The recessive and other behavior recalls the parallel indications from the Pobla de Lillet strain selected for small cell size in which reduction in cell size affected wing size rather than merely the inverse relation between cell size and number. The evidence suggests, therefore, that selection for small cell size has uncovered rather important properties of the genetic variation of body size generally. This topic will be considered in more detail later.

As noted earlier, the various strains and also the cross between the selected strains were reared at 18°C, 25°C and 30°C after five generations of selection. The idea behind this test was as follows. Wing and thorax are highly correlated genetically and reflect variation in total mass. But the genetic correlation is incomplete and the wing-thorax ratio may differ. For example, disproportionate reduction of wing size has been reported in one line in which small wing size was selected for (ROBERTSON and REEVE 1952). In the survey of wild populations dealt with in Table 4 one stock—Gabarros—has relatively bigger wings than the others which are much alike, while we have just considered the case of the difference in wing-thorax ratio between the small cell strain and the control Gabarros stock. In all these instances the differences in relative wing size has been correlated with changes in cell size, just as the changes in wing-thorax ratio affected by rearing larvae at different temperatures are associated with changes of cell size. Nevertheless, the most notable property of the wing-thorax ratio is its comparative constancy within and between populations, under given conditions, as might be expected since there is likely to be an optimum relationship with respect to flying ability. Stability of the relationship between temperature during growth

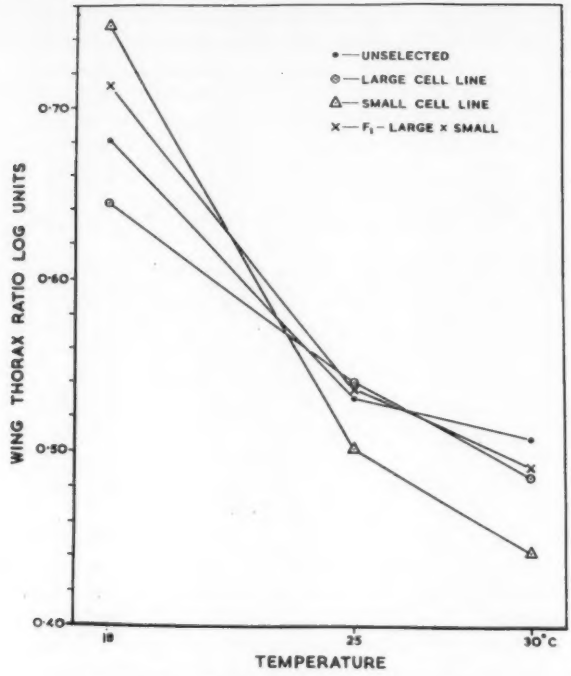

FIGURE 6.—Wing-thorax ratio and temperature.

and the wing-thorax ratio, presents an aspect of genetically controlled regulation of organ size and genotypes probably differ in this respect. Change in the cell size and number relations in the organ concerned, seems particularly likely to interfere with the processes concerned in maintaining the typical relationship.

This appears to be the case. Figure 6 shows the curves relating the wing-thorax ratio (log wing area means log squared thorax length) and temperature for selected lines and their F_1 as well as the unselected stock. The unselected and large cell strains are rather similar in response, whereas the small cell strain is clearly different, especially at 18°C at which temperature the wing-thorax ratio is considerably greater than in the unselected stock, although at 25°C it is lower indicating that the difference in ratio, noted at the end of the selection experiment had been established quite early. The cross is like the unselected and large strains at 25°C and 30°C, although at 18°C the ratio is shifted in favor of that characteristic of the small cell line. It appears therefore, that a shift in favor of large cells and fewer of them is compatible with the normal growth of the wing in relation to body size at different temperatures, whereas the reciprocal type of change is not.

DISCUSSION

As noted in the introductory statements only part of the available data have been dealt with in this paper, which will be followed by an account of what happens to cell size and number when body size is altered by different kinds of genetic change. Hence a complete discussion must await these additional results. However, the present data point to a number of inferences which can now be considered.

Since only part of the body has been studied, generalizations about changes in cell size and number in other parts of the body have to take account of the manner of growth peculiar to this organ. On embryological grounds it is reasonable to assume that adult size is determined by the time the larva pupates. But WADDINGTON (1940) and others have shown that considerable growth, including cell division, accompanies the process of differentiation of the wing and other exoskeletal parts in the pupal period. Although the particular kinds of change in cell size and number in the wing which accompany a given change of body size may also occur in other parts of the body derived from imaginal discs, there is no *à priori* reason why this should be so and only further work can settle this interesting point. This consideration, however, does not affect the value of changes of cell size and number in the wing as indicators of different kinds of genetic and environmental effect.

With respect to environmental variation which affects wing and body size there is a substantial increase in body size as the temperature during growth is lowered from 30°C to 18°C; between these limits wing area increases about 45 percent. As IMAI (1934) noted, body proportions do not remain unchanged at different temperatures; in particular wing area changes 2–2½ times the rate at which the square of thorax length changes. Such a well marked reaction probably has adaptive significance which might be sought in the relations between body mass, rate of wing beat, wing area and prevailing temperature. Comparison of the performance of a number of inbred lines and crosses showed that these considerable changes in wing area are apparently effected almost entirely by change of cell area. Clear-cut gene-environment interaction occurred in the degree of response to a given change of temperature. Although all genotypes followed the same general pattern of change via cell size, they differed in how much wing size was increased or decreased by the same change of temperature. As a result the size relations between say a pair of inbred lines and their F_1 may be drastically altered by changing the temperature a few degrees; and it was noted that such behavior would confer limited significance on formal estimates of dominance deviations derived from parent, F_1, F_2 and backcross data. There is some indication that crosses between inbred lines show a greater response to a decline of temperature from 25°C to 18°C, i.e., they increased in size proportionately more than their parents. At 30°C wing size was smaller than expected in some of the inbred lines, due to a reduction of cell number. Thus a shift from 25°C to 30°C reveals the greater resistance of crosses to extreme conditions while the change from 25°C to 18°C draws attention to their greater responsiveness to environ-

mental change; no doubt both features are ultimately attributable to their greater capacity for homeostatic adjustment during growth.

In the comparisons of the Gabarros strains selected for large and small cell size, thorax length was also recorded and it was possible to compare the wing-thorax relationship or relative wing size at different temperatures. The strain selected for large cells and the unselected strain were alike while the small strain differed, suggesting that the alteration of the cell size and number relations in favor of smaller cells and more of them may provide a more effective way of altering the processes which control the characteristic relationship between wing and general body size at different temperatures than the reciprocal type of change.

Variation in the chemical composition and quantity of food almost certainly accounts, apart from temperature fluctuation, for the greater part of the environmental variation to which *D. melanogaster* is customarily exposed. The interrelations between genotype, nutritional requirements and pattern of development may be explored by finding the effects on cell size and number of different, defined suboptimal diets. It is known (SANG 1956) that the reduction of specific nutrients below a certain level reduces body size and lengthens the period of larval development. Since the minimal requirements of such nutrients are not absolute, but are bound to vary according to the composition of the rest of the medium (BEGG and ROBERTSON 1950), an almost indefinitely large variety of alternative suboptimal diets could be tested. Casein deficiency almost certainly involves deficiency of at most a few essential amino acids. Although slow growth to small size can generally take place in the absence of RNA, indicating a limited ability to synthesize the constituent purines and pyrimidines (SANG 1957) additional RNA is generally necessary for normal growth. Choline is an essential nutrient; since its chief function is probably the provision of methyl groups, various processes of metabolism are probably affected by suboptimal levels. Thus, although shortages of any of these nutrients may lead to a similar reduction in body size, it is likely that different metabolic processes constitute limiting factors.

In spite of this fact, the general response to such different treatments on the part of individuals of the three populations tested is much the same, although the different populations are not identical in their response to the same treatments. There is a strong indication that adverse nutrition which reduces body size and hence wing size, does so by reducing the number of cells while cell area tends to be affected only with more extreme treatments. The same conclusion was drawn from the zero covariance between wing area and cell area with respect to within-culture variance of the crosses between inbred lines, while the nutritional differences between the two tests with the line N_2 were also associated with change of cell number; other unpublished observations have provided additional evidence of this behavior. Hence, provided nutritional variance is not too great, cell area appears comparatively stable.

Clear evidence of the regulation of body size when the larval diet is inadequate has been reported by SANG (1958). For example, a suboptimal supply of protein in the larval diet leads to a lengthening of the larval period, without reduction

of body size below the level attained on an adequate diet. But with further reduction of the protein level a point is reached where body size declines. Genotypes differ with respect to their ability to regulate body size on suboptimal diets (ROBERTSON, in press). It is likely that the tendency for cell size to remain constant in spite of nutritional variation is related to this capacity for regulation.

The contrast between the effects of temperature, which, apart from the special case of the inbred lines at 30°C. are associated with changing cell size and constant cell number and the effects of adverse nutrition, shows how different kinds of environmental change, which may lead to an equivalent difference of body size, may produce these effects in quite different ways. It follows that genetic changes which influence temperature sensitive processes or the ability to utilize particular diets may betray such difference in the cell size and number relations.

The genetic effects which have been studied under favorable conditions of nutrition at a constant temperature may be considered next. Estimates of the total genetic variance of cell size and number in wild populations from comparisons of the variance of genetically uniform crosses between inbred lines suggests that most of the individual variation is genetic in origin. It may be inferred that the variation of wing size is predominantly associated with variation of cell number, although there is an appreciable genetic correlation between wing and cell size as well. In addition there is a substantial contribution from effects which involve an inverse relation between cell size and number i.e. genetically different individuals of the same wing size may differ in cell size and number. Selection on three different populations showed that it was possible to shift the cell size and number, within limits, without affecting wing size.

It might at first seem rather odd that cell size in the wing should vary so much. It must be remembered that the cells are completely flattened so that cell "size" refers rather to surface area than to volume and hence different degrees of stretching would appear as variation of cell area. However, since there is a distinct association between the length of the little bristles carried by the cells and their area, it is rather improbable that such mechanical deformation is very important. The possibility of varying levels of polyteny cannot be ignored. Many insect tissues have highly polytene cells, and although it is generally accepted that imaginal disc nuclei are diploid (KURNICK and HERSKOWITZ 1952), it is conceivable that polyteny, as in the wing scales of Ephestia (HENKE and POHLY 1952) might arise during the pupal period of growth. DOBZHANSKY (1929) showed a high correlation between cell size and estimates of chromosome volume, in a series of genotypes with atypical chromosome number. Work with other animals, notably that of FANKHAUSER (1945) with amphibia, has shown that polyploid cells are larger but this does not affect final organ size due to regulation, which results in an inverse relation between cell size and number when diploid and polyploid types are compared. Apart from these general observations little can be said at present about the basis for the apparent differences in cell size, which will have to be studied biochemically.

The genetically determined processes which stabilize body size in a population

about a characteristic mean are such as to reconcile genetic variation with comparative phenotypic uniformity. The variation of the cell size and number relations probably demonstrates genetic differences in growth which are regulated to ensure stability of final size. It is to be expected that populations will differ in the degree of latitude in this respect as the selection experiments suggested. Thus in the Pacific population there was a striking shift in the cell size and number relations without any change of thorax or wing size. In the Pobla de Lillet population, selection for large cells lead to an inverse change in cell number, and only a slight decline in wing size, which may have been due to linkage or sampling, while in the Gabarros test, selection for large cells also indicated considerable scope for changing the cell size and number relations without affecting wing size. With selection for small cell size however, the picture is rather different, since in both the Pobla de Lillet and Gabarros tests, continued selection lead to a decline in wing size. It appears therefore that the scope for shifting the allocation of materials for growth in favor of smaller cells and more of them is very much less than the reciprocal change. It appears, therefore, that selection for small cell size provides a direct way of selecting gene combinations which destroy the conditions which favor the stability of body size. Strains selected for small cell size are likely to prove useful tools for the genetic study of stabilizing selection (SCHMALHAUSEN 1949) and of the regulation of body size generally; further work along these lines is in progress.

This inference is supported by the purely genetic evidence. Both in the Pobla de Lillet and, especially, in the Gabarros test, the smaller wing size which results from the selection of small cell size behaves in a recessive manner. In the latter case, the average thorax size of the unselected stock and of the F_1 of the cross between it and the small cell strain almost coincide. Previous experience of backcrossing strains selected for small body size to the unselected population (ROBERTSON and REEVE 1954) would lead us to expect a positive departure from intermediacy in the F_1, but this entirely recessive behavior is unprecedented. This suggests that selection for small body size on the one hand, and selection for small cell size on the other, involve different kinds of developmental change which are correlated with differences in genetic behavior. If the selection for small cell size has led to gene arrays which undermine the stability of body size, it is not surprising that such effects should behave as recessive, or perhaps, to use a more exact term, hypostatic to the more normal gene arrays contributed by the unselected parents. Such behavior recalls the property of genetic differences which characterize lines long selected for small body size that have apparently reached fixation (ROBERTSON 1954, 1955). On the earlier evidence it was suggested that continued selection for small body size, eventually breaks up and discards epistatic combinations which favor more normal size in favor of hypostatic effects, thereby accelerating the progress to fixation. It is worth noting that the immediate return to normal size when selection was relaxed in the Gabarros small cell strain is not inconsistant with interaction between a number of genes, whose joint effect is dissipated by segregation. These considerations pose a query as to

the status of the genetic correlation between wing and cell size in the wild populations. This point will be taken up in the next paper of this series in the light of further evidence.

Thus the record of cell size and number in relation to organ and body size brings various dynamic aspects of growth within reach of experimental modification and genetic study. The inference that differences in genetic behavior may be associated with characteristic differences in development points to a more sophisticated analysis of genetic variation which will lead to a synthesis between the concepts of population and developmental genetics—two disciplines which are maintained in a state of unprofitable isolation.

SUMMARY

1. The effects of different kinds of genetic and environmental variation on wing size in *Drosophila melanogaster* have been studied in terms of the changes in cell size and number in the wing. Each wing cell carries a small bristle and so average bristle density in the wing membrane provides the estimate of cell size. Since wing and body size are highly correlated, wing area can be taken generally as a measure of body size. Hence records of wing and cell area, converted to a logarithmic scale, provide the basic data.

2. The higher the temperature during larval growth the smaller the wing and body size and such variation is expressed almost entirely by changes in cell area alone. This was demonstrated by rearing a number of inbred lines and crosses at 18°C, 25°C and 30°C. In addition, the following points were noted:

(a) In inbred lines cultured at 30°C there is often a disproportionate reduction of wing size due to fewer cells; the crosses are unaffected by the suboptimal temperature in this way.

(b) There is a tendency for crosses to show a greater range of cell and wing size between 25°C and 18°C than their inbred parents.

(c) Genotype differ in the relative effect on wing size of culturing larvae at two different temperatures and so the relation between F_1 and parent size may vary considerably according to the temperature during growth.

3. The effects of inadequate nutrition in reducing body size have been studied by rearing larvae of different wild stocks on chemically defined aseptic media, deficient in alternative essential nutrients. Provided the diet is not too unfavorable reduction of wing size is accompanied by change in cell number while cell size remains comparatively constant. But with more extreme conditions cell size is reduced as well.

4. Average thorax length, wing and cell area and cell number were determined on seven unrelated wild populations which differed in average size. Differences in cell number account almost entirely for the difference in wing size and between the populations.

5. The relative contribution of genetic segregation and within-culture environmental variance to the total phenotypic variance of wing size, cell size and cell number in several wild populations, was estimated by comparing the variance

components with those derived from a number of genetically uniform crosses between inbred lines. Almost 70 percent of the variance of wing, cell size and cell number is genetic in origin. In wild populations there is almost always a correlation between wing and cell area which accounts for a phenotypic regression of wing on cell area of about 0.2 in log terms. This is entirely genetic since the environmental covariance is zero and the genetic regression works out at 0.3. Although most of the genetic variance of wing and presumably body size is associated with variation in cell number, an appreciable fraction is associated with variation in cell size.

6. Genetically different individuals of the same wing size may differ in cell size and number. Variation in such inverse relations between cell size and number makes an important contribution to the total genetic variation of cell size, and is regarded as tangible evidence of genetic differences in development which are compatible with stability of final wing and body size.

7. Genetic variation in the cell size and number relations, apparently independently of wing size, has been clearly demonstrated by selection for large and small cell size in three different wild populations. Heritabilities ranged from about 0.2 to 0.5. It proved easier to shift the relations in favor of large cells and fewer of them than *vice versa*. It proved impossible to make a permanent increase in body size by selecting for larger cells whereas selection for small cell size lead to a decline.

8. The genetic effects responsible for small body size in a strain, selected for small cells, behaved as completely recessive or hypostatic in a backcross to the unselected stock. Such behavior contrasts sharply with the relatively more additive behavior in similar crosses when the small parent is the product of selection for small body size. Hence different criteria of selection may apparently lead to the same results—in terms of body size—but, in reality, may affect different developmental processes and involve different kinds of genetic behavior. It is suggested that selection for small cell size provides a direct way of breaking up epistatic combinations which normally ensure the stability of body size in the population.

9. The inference that differences in genetic behavior may be associated with characteristic differences in development points to more informative methods of analysing genetic variation and eventual synthesis of concepts derived from the fields of both population and developmental genetics.

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INHERITANCE AND ASSOCIATION OF OIL AND PROTEIN CONTENT AND SEED COAT TYPE IN SESAME, SESAMUM INDICUM L.[1]

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SINCE considerable progress has been made in the development of disease-resistant indehiscent sesame varieties adapted to mechanized harvesting, breeders will probably concentrate more effort on other agronomic and chemical characteristics of this plant. A knowledge of the genetic behavior of oil content. protein content and seed coat type should be helpful in the selection of high oil and protein lines.

The purpose of this paper is to report inheritance studies of oil content, protein content and rough and smooth seed coat type along with the associations which exist among these characters.

Information pertaining to the inheritance of oil content, protein content and seed coat type in sesame is limited. KINMAN and STARK (1954) reported oil and protein content of the seed of 24 varieties grown at College Station, Texas, in 1950. The range in oil and protein content for varieties was 45.29 to 63.38 and 16.69 to 27.25 percent, respectively, indicating the presence of a rather high degree of variability in the expression of these two characters. The oil and protein content of eight varieties grown at ten locations were given. The mean oil content for varieties exhibited a range of 47.34 to 55.45 percent and for locations a range of 50.63 to 56.58 percent. The mean protein for varieties exhibited a range of 25.09 to 28.21 percent and for locations a range of 22.24 to 29.49 percent. These data indicated that oil and, to a lesser extent, protein content of sesame seed are markedly influenced by both genotype and environment. They suggest that the genetic and environmental influences affecting protein content inversely affect oil content and that protein synthesis is favored over oil synthesis as the nitrogen supply to the seed increases.

CULP (1953), in a preliminary study of the inheritance of oil content and seed coat type in sesame, found that seed coat type was simply inherited, being conditioned by one pair of genes, and that two to seven polygenes were involved in the inheritance of oil content. Rough seed was found to possess lower oil content than smooth seeded segregates. This is due to the fact that the rough seed coat makes up a larger part of the total seed weight than the smooth seed coat. (It

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should be noted that rough seed coat has no economic importance in sesame breeding at the present time but was used as a genetic marker in these inheritance studies.) The F_1 generation was found to be intermediate in oil content. There was some evidence for partial dominance of low oil content; however, this may have been due to the influence of the rough seed coat type. These data indicated that at least two different types of factors were responsible for the segregation of oil content in sesame. These two factors were the single pair of genes conditioning seed-coat type which influenced the amount of oil-bearing tissue and the polygenic complex conditioning the inheritance of oil in the oil-bearing tissues of the seed.

MATERIALS AND METHODS

Parental material selected for this study consisted of two lines of sesame, K8-4-5-1-2 and N124-9-3-6-2-3-5-2, hereafter referred to as K8 and N124. K8 was chosen for its high oil content, lower protein content and smooth seed coat type. N124 was chosen for its low oil content, higher protein content and rough seed coat type. These two parents were considered to be in a homozygous condition for the characters under study, since they had been selfed for a number of generations.

During the fall and winter of 1951–52, seed of the F_1, F_2, both backcross generations and selfed seed of the respective parents were obtained in the greenhouse. These progenies and parents were grown in a randomized block design of three replications on a Norwood clay soil at College Station, Texas, in 1952. The planting was made in rows 17 feet long and 40 inches apart with six inches between adjacent plants within the rows. Each plant was harvested as an individual selection, and seed-coat type, percentage germ (decorticated or hulled seed), oil content and protein content of the whole seed and calculated oil and protein content of the germ were determined.

In order to produce a representative sample of the F_3 generation, seed from 180 F_2 plants (135 rough seeded and 45 smooth seeded segregates) were planted at College Station, Texas, in 1953. No statistical design was used for this study. The planting was made in three ranges of 60 rows each, 17 feet long with 40 inches between rows. The F_3 lines were classified according to segregation for seed coat type, and 10 to 15 plants were harvested as a representative bulk sample of each F_3 line. Percentage germ, oil content and protein content of the whole seed and calculated oil and protein content of the germ were determined for each sample.

Seed from plants of the F_1, F_2, F_3, backcross generations and respective parents were decorticated and the percentage germ of the whole seed was determined. The term "percentage germ" refers to the percentage of decorticated or hulled seed obtained from a sample of whole seed. (No attempt was made to remove the small amount of endosperm present in these samples.) A one gram sample of seed was soaked in about 40 mls of 1.0 N NaOH solution for one to two minutes. The NaOH solution was then decanted and replaced with approximately the same amount of water. This solution was made slightly acid by the addition of 1.0 N HCl solution,

using phenolphthalein as an indicator. The seed coats were rubbed from the seeds by using a Sargent wet mill which had been modified by placing a rubber tube over the central grinding wheel to reduce seed breakage and crushing. The seed coats were removed from the seeds in about three minutes. As much of the seed coat as possible was washed from the sample before it was placed on a filter paper in an oven and dried at 100°C. After the sample was dry, fragments of the seed coat were removed by hand, and the sample was weighed. Percentage germ was then calculated as the proportion of decorticated seed obtained from the sample of whole seed.

Seed coat type was determined visually in most cases; however, some samples were difficult to classify by this means. Classification of these samples was made by rubbing a small amount of seed in the palm of the hand. When the seed was rubbed the seed coat of the rough type was removed, but the seed coat of the smooth type remained intact.

Samples were prepared for oil content determination by grinding a two gram sample of seed with a mortar and pestle. The ground material was then extracted with petroleum ether for four hours on a Goldfisch fat extractor, and the weight of the extract was reported as percentage oil on an air-dry basis.

Percentage protein of the seed was calculated by determining the total nitrogen in a 1.75 gram sample of seed using the Kjeldahl-Wilfarth-Gunning method (A.O.A.C. 1950), and by converting total nitrogen to crude protein by using a multiplicative conversion factor of 6.25.

In order to determine whether any oil or protein was carried in the seed coat, the seed coat was removed from 20 rough-seeded samples of the F_3 generation with a modified Buckeye barley pearler. Oil and protein content of the seed coat were determined in a manner similar to that described above.

Oil and protein content of the germ were determined as the ratio of oil or protein content of the whole seed to percentage germ of the seed.

Analyses of variance were made as outlined by SNEDECOR (1946). Scaling tests were made and the variance was partitioned into the fixable genetic (D), unfixable genetic (H) and nonheritable components (E) as outlined by MATHER (1949). Correlation and regression coefficients for all pairs of characters were calculated by the methods outlined by SNEDECOR (1946). Genetic correlations were estimated using the method suggested by HAZEL (1943). Estimates of heritability were calculated using the different methods suggested by LUSH (1948), WARNER (1952), and BURTON (1951). The number of genes was estimated using various formulas given by MATHER (1949), CASTLE and WRIGHT (1921), WEBER (1950), and BURTON (1951).

EXPERIMENTAL RESULTS AND DISCUSSION

The two sesame lines used as parents in this study of quantitative and qualitative inheritance were representative of the most divergent types available for percentage germ and oil of the whole seed. Mean differences between parents for percentage germ, oil and protein of the whole seed were 8.40, 7.18 and 1.11 per-

cent respectively. Except for the small mean difference in protein content of the whole seed, it was assumed that all the characters possessed acceptable differences for inheritance studies of polygenic traits. This assumption is supported by analysis of variance based on generation means. The mean squares for all characters except protein content of the whole seed were significant or highly significant. No significant differences were found between replications.

Segregation of characters

Seed coat type and percentage germ: Percentage germ of the whole seed as calculated in this study is the remaining complement of the seed after removal of the seed coat. The amount of seed coat is highly dependent on whether the seed coat is rough or smooth. The presence of either rough or smooth seed is apparently simply inherited and controlled by one pair of genetic factors. The F_1 and BC_1 ($F_1 \times$ N124) plants possessed rough seed, indicating dominance of this character. The F_2 generation of 252 plants consisted of 185 rough and 67 smooth seeded plants. This segregation conforms to an expected 3:1 ratio (Chi-square = 0.339). The BC_2 ($F_1 \times$ K8) of 112 plants was composed of 49 rough and 63 smooth seeded segregates. This conforms to an expected 1:1 ratio (Chi-square = 1.750). In the F_3 generation grown from 135 rough and 45 smooth seeded F_2 plants, all the smooth seeded plants produced smooth seeded offspring. The 135 rough seeded plants gave 61 rough seeded lines and 74 lines segregating for seed coat type. These data did not conform to the expected 1:2 ratio (Chi-square = 8.533). This can probably be attributed to sampling error in choosing the F_2 plants for the production of the F_3 lines. The symbol *rr* is suggested as the designation for the smooth seed factors.

Although the presence of rough or smooth seed coat type is simply inherited, being conditioned by one pair of genes, the inheritance of the amount of seed coat is much more complex. The inheritance of the amount of seed coat as such was not investigated, but percentage germ of the whole seed was studied.

The ranges, means, midparental values and standard errors of percentage germ of the parents and generations are presented in Table 1. The wide ranges in percentage germ of the parents and F_1 generation indicate that this character is highly influenced by environment. The means of all generations are lower than expected when the midparental values are used as the expected values. These data indicate either that partial dominance of low percentage germ exist or that rough seed coat is associated with low germ percentage.

Oil content: The ranges, means, midparental values, and standard errors of oil content of the whole seed of the parents and generations are shown in Table 1. The wide ranges in oil content of the parents and F_1 generation indicate that oil content is highly influenced by environment. The means of the F_1, F_2 and backcross generations are lower than expected with respect to the midparental values. Since low germ percentage may be associated with rough seed this indication of partial dominance for low oil content may be attributed to the presence of rough seeded plants.

Since it was believed that the type of seed coat present exerted an influence on

TABLE 1

Ranges, means, standard errors and mid-parental values for the five characters under study in the sesame cross, N124 × K8

Character	Parent or cross	Number of plants	Range	Mean	S.E.	Mid-parental value
Percentage germ	P_1	55	77.7 –85.7	82.4	.25	....
	P_2	66	84.2 –94.7	90.8	.28	....
	F_1	44	81.0 –89.9	84.3	.26	86.6
	F_2	212	75.5 –93.0	84.7	.24	86.6
	BC_1	108	77.7 –87.6	83.2	.19	83.4
	BC_2	105	76.3 –93.9	87.0	.38	87.6
Percentage oil of whole seed	P_1	62	40.33–47.62	44.00	.22	....
	P_2	67	48.82–55.20	51.18	.20	....
	F_1	54	42.59–49.87	46.39	.25	47.59
	F_2	236	41.38–56.16	46.76	.17	47.59
	BC_1	126	39.50–49.57	44.97	.17	45.20
	BC_2	110	41.46–53.68	47.74	.24	48.78
Percentage oil of the germ	P_1	55	49.36–55.96	53.08	.25	....
	P_2	66	53.12–60.36	56.34	.22	....
	F_1	44	51.56–57.55	54.96	.25	54.71
	F_2	212	45.98–62.79	55.09	.18	54.71
	BC_1	108	50.50–57.34	54.11	.15	54.02
	BC_2	105	47.71–61.13	54.77	.26	55.65
Percentage protein of the whole seed	P_1	62	25.12–29.28	27.28	.12	....
	P_2	67	23.80–28.04	26.17	.12	....
	F_1	54	24.96–28.64	26.59	.13	26.72
	F_2	236	22.40–31.78	26.90	.12	26.72
	BC_1	126	24.00–30.16	26.82	.11	26.93
	BC_2	110	21.24–30.16	26.90	.16	26.38
Percentage protein of the germ	P_1	55	30.23–34.90	33.28	.14	....
	P_2	66	25.55–32.43	28.85	.19	....
	F_1	44	28.97–35.10	31.51	.21	31.06
	F_2	212	25.36–36.53	31.71	.14	31.06
	BC_1	108	27.66–35.82	32.04	.16	32.40
	BC_2	105	26.47–35.54	30.99	.18	30.18

the inheritance of percentage germ, oil and protein, it should be helpful in this study if the effects of seed coat type were removed. By using percentage germ of the whole seed and oil and protein percentage of the whole seed, it was possible to calculate percentage oil and protein on the basis of percentage germ of the seed. In order to make these calculations it was necessary to assume that all the oil and protein in the seed was carried in the germ. The mean oil and protein content of the seed coat of 20 rough seeded F_3 lines was 1.13 and 3.72 percent, respectively. These values may be higher than the true values since the barley pearler used to decorticate these samples may have removed some tissue other than seed coat. These data indicate that oil content of the seed coat was very close to zero, but a

small amount of protein was carried in the seed coat. Nevertheless, it was assumed that no gross errors were made in converting oil and protein content of the whole seed to oil and protein content of the germ.

The ranges, means, midparental values, and standard errors of oil content of the germ of the parents and generations are given in Table 1. These data indicate that converting oil content of the whole seed to oil content of the germ reduced the variability between the parents and segregating progenies. The means of the segregating generations were slightly higher than the expected midparental values. Thus the indications of partial dominance of low oil content can be attributed to rough seeded segregates which were usually lower in oil. When the effects of the rough seed coat were eliminated, there was some evidence for partial dominance of high oil content.

Although the ranges of the high and low oil parents overlapped after converting oil content of the whole seed to oil content of the germ, the means of all generations are very close to the expected midparental values. It is evident that the low oil parent carried factors for high oil content and the high oil parent carried factors for low oil content.

Protein content: The ranges, means, midparental values and standard errors of protein content of the whole seed of the parents and generations are given in Table 1. The mean difference in protein content of the whole seed between the parental lines was only 1.11 percent. Apparently a high protein line of K8 was chosen as the low protein parent by chance since KINMAN and STARK (1954) reported the protein content of N124 and K8 as 25.62 and 18.44 percent, respectively. Although the mean difference in protein content between the parents is very small, it is interesting to note that the means of the F_1 and F_2 generation are almost midway between the parental means. Transgressive segregation of protein content is evident.

The ranges, means, midparental values and standard errors of protein content of the germ of the parents and generations are presented in Table 1. Coverting protein content of the whole seed to protein content of the germ increased the difference between parental means by 3.32 percent, a statistically significant difference. The F_1 mean was almost midway between the parental means and the F_2 mean approximated the F_1 mean. There is some evidence for partial dominance of high protein content since the observed means of most of the generations were slightly higher than the expected midpoint values.

Associations in the F_2 generation

Correlation and regression coefficients for pairs of characters in the F_2 generation are presented in Table 2. The correlation and regression coefficients indicate a highly significant positive association of percentage germ with oil (r=.531, b=.360) and protein (r=.337, b=.166) content of the whole seed. The correlation and regression coefficients for percentage germ paired with oil and protein percentage of the germ indicate a highly statistically significant negative association between percentage germ and these two characters.

TABLE 2

Correlation coefficients, regression coefficients and genetic correlations for pairs of characters of F_2 plants and F_3 lines of the sesame cross N124 and K8

Characters correlated	F_2 Generation		F_3 Generation		Genetic correlation coefficient	
	Correlation coefficient	Regression coefficient	Correlation coefficient	Regression coefficient	Observed	Estimated
Percentage germ and percentage oil of the whole seed	.531**	.360**	.587**	.396**	1.030	.806
protein of the whole seed	.337**	.166**	.268**	.124**	.512	.308
oil of the germ	—.311**	—.218**	—.304**	—.201**	.000	—.158
protein of the germ	—.314**	—.179**	—.339**	—.197**	—.232	—.127
Percentage oil of the whole seed and percentage protein of the whole seed	—.131	—.095	—.194	—.134	—.056	—.118
oil of the germ	.640**	.660**	.588**	.578**	.000	.023
protein of the germ	.478**	.402**	—.532**	—.445**	—.535	—.184
Percentage protein of the whole seed and percentage oil of the germ	—.449**	—.637**	—.575**	—.821**	—.912	—.143
protein of the germ	.787**	.910**	.793**	.964**	.000	.000
Percentage oil of the germ and percentage protein of the germ	—.249**	—.203**	—.261**	—.222**	.000	.000

** Indicates significance at the 1 percent level.

Oil percentage of the whole seed exhibited a high degree of association with oil and protein percentage of the germ. A negative correlation between oil and protein content of the whole seeds was expected, but no such relationship was established in this study. A highly significant negative relationship was exhibited by the correlation and regression coefficients involving oil and protein content of the germ. Although the correlation and regression coefficients were highly significant in most cases, the magnitude of these coefficients throughout this study was expected to be greater than was actually observed.

Associations in the F_3 generation

Correlation and regression coefficients for pairs of characters in the F_3 generation are given in Table 2. The correlation and regression coefficients exhibited the same character associations as those observed in the F_2 generation. The magnitude of the correlation and the regression coefficients in the F_3 generation was larger in most cases than corresponding values in the F_2 generation. This suggests that the mean of an F_3 line provides a better measure of the genotype than does that of an individual F_2 plant.

Associations in the F_2 and F_3 generation

Correlation and regression coefficients between the values for given characters of 157 F_2 plants and the means for the same characters in their F_3 progenies are presented in Table 3. Although all of the correlation and regression coefficients except those for oil percentage of the germ were highly significant, the magnitude

TABLE 3

Correlation and regression coefficient for given characters of 157 F_2 plants and for the same characters of their F_3 progeny rows from the sesame cross, N124 and K8

Characters correlated	Correlation coefficients		Regression coefficients	
	Observed	Estimated	Observed	Estimated
Percentage germ	.513**	.734	.483**	.690
Percentage oil of the whole seed	.464**	.591	.434**	.496
Percentage protein of whole seed	.338**	.564	.299**	.579
Percentage oil of the germ	—.038	.470	—.034	.404
Percentage protein of the germ	.252**	.586	.234**	.546

** Indicates significance at the 1 percent level.

of these values was lower than expected. The small correlation and regression coefficients as well as the negative correlation and regression coefficients for oil content of the germ are attributed to experimental error.

Since the correlation and regression coefficients were unexpectedly low and oil percentage of the germ gave negative coefficients, correlation and regression coefficients were estimated by the following formulas. These formulas were de-

$$r_{F_3/F_2} = \frac{1/2D + 1/8H}{\sqrt{1/2D + 1/4H + E_1 \times 1/2D + 1/16H + E_2}}$$

$$b_{F_3/F_2} = \frac{1/2D + 1/8H}{\sqrt{1/2D + 1/4H + E_1}}$$

rived by substituting the components of variation (MATHER 1949) for the covariance, F_2 and F_3 variances. These formulas should give reliable estimates since the D, H and E values were calculated using the progenies of all generations of the cross whereas the correlations were obtained from a portion of the F_2 generations along with the respective F_3 means. Experimental errors which would account for negative and low coefficients were reduced by using these formulas.

The estimated correlation and regression coefficients are presented in Table 3 for the five characters under study. The correlation and regression coefficients as estimated by the above formulas were about 20 to 40 percent greater than observed coefficients and may be of more practical value to plant breeders than the actual values obtained.

Genetic and environmental associations

Genetic correlations were estimated using the formula given by HAZEL (1943) and HAZEL, *et al.* (1943). This formula provides an estimate of the genetic correlation between a pair of characters, eliminating most of the dominant and epistatic deviations and all the environmental deviations.

The observed and estimated genetic correlation coefficients for characters in the F_2 and F_3 generations are presented in Table 2. Two estimates of the genetic correlations were possible since observed and estimated regression coefficients

were available for the same characters in the F_2 and F_3 generations. Although a comparison of the observed correlation coefficients between characters in the F_2 and F_3 generations has been made, it is of interest to compare them with the genetic correlation coefficient. In general, when the F_2 and F_3 observed correlations were large, the magnitude of the genetic correlations was also large. This did not hold true for the association between oil percentage of the whole seed and oil percentage of the germ or for that between protein percentage of the whole seed and protein percentage of the germ. In most cases, the genetic correlations of percentage germ with oil and with protein percentages of the whole seed for germ were large, whereas genetic correlations between oil and protein percentage of the whole seed or germ were small.

The genetic correlation coefficients calculated using estimated regression for the same characters in the F_2 and F_3 generations were in most cases much smaller than expected. This can be attributed to the method of calculating these values. There was no way to adjust the values which made up the numerator of the formula. Because of this, the genetic correlations are perhaps underestimated in most cases.

Components of variation

Partitioning of the variance into the four components, fixable genetic (D), unfixable genetic (H), nonheritable within plots (E_1), and nonheritable between plots (E_2) for all characters studied was conducted as outlined by MATHER (1949). The mean variance for the three replications was used wherever possible. Direct estimates of E_2 were calculated, using the five rows of the F_2 generation in each replication. Only one estimate of the covariance (W_{F_3/F_2}) was available, since the F_3 generation was not replicated. The D, H, E_1, and E_2 values for the five characters are given in Table 4. The D values for the two characters, percentage germ and percentage oil of the whole seed were highly significant. The E_1 value for percentage oil of the whole seed was significant at the five percent level of probability. The D and E_1 values for the other characters approached significance at the five percent level of probability. Standard errors of all determinations were very large. This may be at least partially attributed to having only two degrees of freedom by which to divide the sum of squares of the deviations of the observed variances from that of the expected variances. The unfixable genetic variance (H) for percentage germ, protein percentage of the whole seed and protein percentage of the germ were negative but not significant. These data indicate that: (1) a large part of the variance is additive or fixable genetic variance, (2) a large part of the variance can be attributed to environmental or nonheritable causes and (3) very little of the variance can be attributed to unfixable genetic factors for the characters under study.

Heritability of characters

Heritability of characters was estimated by methods suggested by LUSH (1948), WARNER (1952), MATHER (1949), and BURTON (1951). These estimates of heritability for each of the characters studied are presented in Table 5.

TABLE 4

D (*fixable genetic variance*), H (*unfixable genetic variance*), E_1 *and* E_2 (*environmental variance*) *values for the characters studied in the sesame cross N124 × K8*

Components	Estimate	Standard error
	Percentage germ	
D	16.7238**	4.2164
H	—3.5934	11.2960
E_1	4.0125**	1.8845
E_2	1.9962	1.6112
	Oil percentage of the whole seed	
D	6.5587**	2.1313
H	1.4959	5.7099
E_1	2.5765*	.9526
E_2	1.0266	.8144
	Protein percentage of the whole seed	
D	3.0751	1.5883
H	—.0088	4.2552
E_1	1.1170	.7099
E_2	.7133	.6064
	Oil percentage of the germ	
D	4.0441	5.4996
H	.5976	46.5932
E_1	3.0159	2.4580
E_2	1.7790	2.1016
	Protein percentage of the germ	
D	4.0274	2.0594
H	—.9479	5.5173
E_1	1.6942	.9204
E_2	1.0604	.7875

* Significant at the 5 percent level.
** Significant at the 1 percent level.

The estimates using regression coefficients were lower than expected for all characters. This is due to the small magnitude of the covariance values. Using estimated regression coefficients the values of heritability for all characters were increased to within the range of the other estimates. The high estimates obtained using WARNER's method (1952) may be attributed to the variance of the F_2 being greater than expected in comparison to the variance of the BC_1 and BC_2 generations. Heritability estimates using the ratio of $1/2D$, as calculated by MATHER (1949), with either the observed or estimated F_2 variance gave about the same values. The calculation of $1/2D$ using WARNER's method (1952) as $2V_{F_2} - V_{BC_1} + V_{BC_2}$ has more practical application than MATHER's method (1949); however, the chance for error in heritability estimates becomes greater. When $1/2D$ is calculated as suggested by MATHER (1949) and substituted in WARNER's formula (1952), heritability estimates were reduced 10 to 20 percent.

TABLE 5

Heritability estimates for germ, oil and protein content of sesame calculated by different statistical methods

Method	Whole seed			Germ	
	Germ	Oil	Protein	Oil	Protein
†Regression =					
Observed	.483	.434	.299	—.034	.234
Estimated	.690	.496	.579	.404	.546
‡$\frac{2V_{F_2} - V_{B_1} + V_{B_2}}{V_{F_2}}$ =	.859	.638	.870	.815	.727
§$\frac{\frac{1}{2}D}{V_{F_2}}$ =	.663	.499	.459	.326	.489
††$\frac{\frac{1}{2}D}{\frac{1}{2}D + \frac{1}{4}H + E_1}$ =	.729	.526	.580	.413	.580
‡‡$\frac{V_{F_2} - V_{F_1}}{V_{F_2}}$ =	.766	.562	.750	.636	.609
Mean	.712	.521	.592	.445	.556

† LUSH's method (1948).
‡ WARNER's method (1952).
§ WARNER's method (1952) using MATHER's (1949) $\frac{1}{2}D$ and the phenotypic variance.
†† WARNER's method (1952) using $\frac{1}{2}D$ and expected variance.
‡‡ BURTON's method (1951).

Although BURTON's method (1951) of estimating heritability of a character is in the broad sense of the term, such estimates were made for comparison with those obtained by other methods. Since it was shown that the phenotypic variances of the characters were not influenced by unfixable genetic factors, BURTON's method (1951) should give heritability values equal to those obtained by the other methods. It is of interest to note that heritability values calculated using estimated regression coefficients, WARNER's method (1952) using $1/2D$ as calculated using MATHER's method (1949) and BURTON's method (1951) gave heritability values of about equal magnitude. An average of the heritability values obtained with these methods indicates that heritabilities of about 70, 60 and 50 percent can be assumed for the characters percentage germ, protein and oil, respectively. With heritability values of these magnitudes, breeders can expect to make rapid progress in altering percentage germ, oil and protein by selection of superior plants.

Gene number

The number of effective factors determining each character studied (Table 6) was estimated by formulas suggested by MATHER (1949). CASTLE-WRIGHT (1921), WEBER (1950) and BURTON (1951). All the formulas gave about the same results. These data indicate that one or two genes condition each of the

TABLE 6

Estimates of the number of genes conditioning the expression of the characters in the sesame cross, N124 × K8

Character	Estimate	Number of genes			
		†	‡	§	††
Percentage germ		1.08	.93	.97	1.07
Percentage oil of the seed		1.94	1.72	1.45	1.79
Percentage protein of the seed		.11	.07	.06	.07
Percentage oil of the germ		1.01	.53	.50	.57
Percentage protein of the germ		1.29	.96	1.35	1.01

† Calculated using MATHER's method (1949).
‡ Calculated using the CASTLE-WRIGHT formula (1921).
§ Calculated using WEBER's method (1950).
†† Calculated using BURTON's method (1951).

characters under study. These values are probably lower than the true gene number but indicate that only a few factors conditioned the inheritance of the characters in this study. Since heritability estimates were rather large and gene numbers low, rapid progress in altering percentage germ, oil and protein by the process of selection can be expected.

SUMMARY

Segregating progenies of a cross were studied in an attempt to investigate the inheritance of oil and protein content in the presence and absence of rough and smooth seed coat type. Seed coat type was found to be simply inherited. Segregation into rough and smooth seed coat types was controlled by one pair of genes with rough seed coat dominant. The inheritance of percentage germ, oil and protein content was found to be more complex.

Partitioning of the phenotypic variance of each of the characters under study into the component parts indicated that: (1) a large part of the variance was additive or fixable genetic variance, (2) another large part of the variance can be attributed to nonheritable causes, and (3) very little of the variance can be attributed to dominance deviations.

Heritability values of about 70, 60 and 50 percent were found for the characters percentage germ, protein and oil, respectively. The number of genes determining the inheritance of each character was estimated as one or two. Since heritability of characters was high and only a few genes are involved for each character, breeders can expect to make rapid progress in altering percentage germ, oil and protein by selection.

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THE DUBININ EFFECT AND THE Y CHROMOSOME[1]

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DUBININ and SIDOROV (1934) found that certain translocations of the fourth chromosome in *Drosophila melanogaster* are associated with a weakened dominance of the wild type allele of the cubitus interruptus gene, ci^+, when they are tested against the *ci* mutant. Previously, it had been established that some genes, notably white and brown (MULLER 1930; GLASS 1933) normally located in euchromatin and brought into heterochromatin through a chromosomal rearrangement, also showed a weakened dominance of their wild type allele. In these latter cases, the action of the gene appeared to fluctuate in its expression from cell to cell or from one group of cells to another in the fly so that a somatic mosacism resulted and the term variegated-type position effect was applied to this class of phenomena (see Review by LEWIS 1950). The question as to whether the **R(ci$^+$)/ci** phenotype is variegated, where $R(ci^+)$ symbolizes the rearranged chromosome, necessarily remains in abeyance since the *ci* mutant is variable in expression and since the character is limited to breaks in a single wing vein. KHVOSTOVA (1939) showed in a comprehensive cytological analysis that the ci^+ position effect is almost invariably associated with a euchromatic-heterochromatic type of rearrangement which involves a break in the proximal heterochromatin of chromosome four, close to the region of the *ci* gene.

The "Dubinin effect," a term often applied to position effects at the *ci* locus, differs from most V-type position effects in that $R(ci^+)$ in the homozygous condition, or when present in one dose either as the hemizygote or when tested over a deficiency is wild type, whereas most $R(+)$ when viable in the homozygote or hemizygote or when tested over a deficiency exhibit a variegated mutant phenotype. DUBININ and SIDOROV (1934) found, however, that $R(h^+)$, a euchromatic locus in 3L which shows a V-type position effect in combination with its mutant allele, hairy, although lethal in this case as a homozygote, is wild type when tested against a deficiency for the hairy locus. Another point of resemblance between $R(ci^+)$ and $R(h^+)$ is that the criterion for phenotypic variegation, i.e., somatic mosacism for the expression of both the mutant and the wild type alleles, cannot be satisfactorily applied in either case.

An important criterion for the recognition of a V-type position effect is the susceptibility of a variegating gene to modification by changes in the euchromatic-heterochromatic balance. GOWEN and GAY (1933) first showed that the variegation of the white gene could be more or less suppressed by adding a Y

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chromosome. Since then, the ability of an extra Y to suppress variegation has been demonstrated for many $R(^+)$ euchromatic loci. Several investigators (PANSHIN 1936; KHVOSTOVA 1939) have attempted to determine the effect of an extra Y on the $R(ci^+)$ position effect but a clear-cut answer has never been obtained. SCHULTZ (1936) discovered that variegation resulting from rearrangements of the normal allele of the light gene, lt^+, which lies within or close to the chromocentral heterochromatin of the second chromosome, is also modified by the addition of an extra Y chromosome. In this case the effect is to enhance the variegation of light. Based on these observations a kind of rule has been formulated which states that variegation of euchromatic loci is suppressed by an extra Y chromosome whereas variegation of genes located within or close to heterochromatin is enhanced by an extra Y (MORGAN and SCHULTZ 1942).

In the experiments described below, the influence of an extra Y chromosome on the Dubinin effect has been measured in order to determine whether the Dubinin effect is modified by an extra Y and thus conforms to the V-type position effects. Furthermore, since the *ci* locus lies within or close to the chromocentral heterochromatin, it is of particular interest to determine whether $R(ci^+)$ constitutes the second case of a position effect which is enhanced by the addition of an extra Y chromosome.

The results obtained in these experiments increase the likelihood that the Dubinin effect is a V-type position effect since for each rearrangement tested a highly significant difference exists between females with and without a Y chromosome. Moreover, the modification of the position effect is, in each case, in the direction of suppression.

GENERAL METHODS AND MATERIALS

The expression of $R(ci^+)/ci$ is subject to modification from a wide variety of sources both environmental and genetic. In order to measure the effect of a single variable, in this case the Y chromosome, it is necessary to have control over any modifier genes. To meet this need methods were devised whereby groups of flies with different numbers of Y chromosomes were obtained from a single mating. These methods are described under Experimental Procedure.

The methods which were used also provided for a measurement of the degree of vein interruption a generation before it was possible to know whether the fly carried a Y chromosome. The determination of the presence or absence of a Y in the following generation was nearly always made without knowledge of the *ci* index ascribed to the parent a generation before. In this way subjective errors, if not eliminated entirely, were considerably reduced.

A complication which arises in working with translocation heterozygotes of the fourth chromosome is the tendency of such stocks to become partially triplo-4 unless they are balanced with a fourth chromosome dominant mutant which is lethal when present in two doses. The translocation will cause some nondisjunction between it and the free four producing diplo-4 and nullo-4 gametes and consequently triplo-4 and haplo-4 individuals. Such triplo-4 individuals will,

through preferential segregation of the two normal fours, be maintained to a greater or lesser degree in the stock. If the normal fourth chromosome of such a translocation stock carries the normal allele of *ci*, then on outcrossing to *ci*, both *R(ci⁺)/ci* and *R(ci⁺)/ci⁺/ci* will be tested. Those translocation bearing progeny which carry one *ci⁺* will not show the Dubinin effect.

Also rearrangements balanced with *ci ey^R* and selected on the basis of non-*ey^R* will tend to become triplo-4 since this condition will cause a shift of venation toward normality (STERN, MACKNIGHT, and KODANI 1946). On outcrossing such a translocation, the great majority of translocation bearing gametes will carry an extra *ci ey^R* fourth chromosome. The effect of an extra Y or X0 condition on *R(ci⁺)/ci/ci* instead of on *R(ci⁺)/ci* will then be measured.

In the present study, the possibility of this complication was avoided by clearing the stocks of extra fourth chromosomes and then rebalancing in a way that eliminates the possibility of the recurrence of this condition.

MATERIALS AND STOCKS

1. T(3:4)86D:Translocation(3:4)86D. This translocation which was found by E. B. LEWIS, was induced by irradiation of *bx^34e e⁴* males with 400 rep units of fast neutrons. The salivary gland chromosome analysis by LEWIS shows a break in 3 just after 86D1–2 and a break in 4 at 101F. The translocation is homozygous viable and the homozygote has normal venation. The translocation was established in a homozygous stock free of extra fours.

2. T(3:4)89E: Translocation (3:4)89E. This translocation which was found by E. B. LEWIS, was obtained from an X-rayed *ss bx Su^s -ss* male. It is inseparable from *bxd^101*, an extreme bithoraxoid mutant. The salivary chromosome analysis by LEWIS shows a break in 3 between 89E1–2 and 89E3–4 doublets and at 101 in chromosome 4. The translocation was balanced against *ey^D* and freed of extra fourth chromosomes.

3. *In(1)*dl-49,*v^0f*/*In(1)*dl-49,*v^0f*/Y^c-s;*ci ey^R*/*ci ey^R* ♀ × +/Y^c-s;*ci ey^R*/*ci ey^R* ♂. The *In(1)*dl-49,*v^0f* chromosome was derived from a single crossover between an *In(1)*dl-49,*v^0f* chromosome and a Canton-S chromosome. Thus, the proximal region of the X chromosome, including the centromere, is of Canton-S origin. The symbol Y^c-s indicates a chromosome from a Canton-S wild type stock.

4. *ci gvl bt*. This stock was obtained as a crossover by A. H. STURTEVANT and maintained as a homozygous stock since 1951.

5. *ci sv^n*. This stock was obtained as a crossover by A. H. STURTEVANT and maintained as a homozygous stock since 1951.

Mutant or rearrangement symbols used in the tests or discussed below are as follows (for fuller description see BRIDGES and BREHME and Drosophila Information Service Nos. 27 and 28):

al² (aristaless²), *B* (Bar), *bt* (bent), *bw* (brown), *bx* (bithorax), *bx^34e* (bithorax ^34e), *bxd^101* (bithoraxoid^101), *ci* (cubitus-interruptus), *dm* (diminutive), *e⁴* (ebony⁴), *ey^D* (eyeless-dominant), *ey^R* (eyeless-Russian), *FM3* (First

Multiple 3), *gvl* (grooveless), *In(1)*dl-49 (Inversion (1) delta-49), *In(2L)Cy* (Inversion (2L) with Curly), *In(2R)bw*^{VDe1} (Inversion (2R) brown-Variegated of Demerec no. 1), *lt* (light), *M(2)S10* (Minute (2) Schultz' no. 10), *SM1* (Second Multiple 1), *Sb* (Stubble), *sc*^{s} (scute^{s}), *ss* (spineless), *Su*^{z}*-ss* (Suppressor^{z} of spineless), *sv*^{n} (shaven-naked), *sp*^{z} (speck^{z}), *v* (vermilion), *v*^{Of} (vermilion of Offermann), *y*^{z} (yellow^{z}), *y*^{31d} (yellow^{31d}), Y^{C-S} (Y chromosome derived from Canton-S stock), *Xa* (Xasta).

Classification

The classification of the degree of cubitus interruption is that used by STERN, SCHAEFFER and HEIDENTHAL (1946). The portion of the fourth vein examined was that distal to the posterior cross vein. A completely normal vein was given a value of 0; a thinning at one or several points but no discernible break was given a value of 1; the absence of the vein for any distance up to a maximum of one half was classified as 2; a break greater than one half and no greater than seven eights the vein length was classified as 3. Retention of one eighth or less was valued at 4.

Each wing was graded as a unit and the two values for each female tested were added. Nine classes were set up extending from 0–8.

EXPERIMENTAL PROCEDURE AND RESULTS

Effect of one extra Y chromosome on R(ci^{+})/ci: Single virgin females whose genotype was *In(1)*dl-49,*v*^{Of}/*In(1)*dl-49,*v*^{Of}Y^{C-S}; *ci ey*^{R}/*ci ey*^{R} were mated in vials to single males which were homozygous for T(3:4)86D or to single males which were heterozygous for T(3:4)89E. The vials were kept at 25°C for 24 hours and then the flies were transferred to bottles which were kept at 20° C until hatching. The regular translocation bearing female progeny of such matings consist of two types which are phenotypically indistinguishable. These are:

Type 1. *In(1)*dl-49,*v*^{Of}/+; T(3:4)/+; *ci ey*^{R}

Type 2. *In(1)*dl-49,*v*^{Of}/+/Y^{C-S}; T(3:4)/+; *ci ey*^{R}

Thus two groups of daughters are obtained from a single mating, one of which carries an extra Y chromosome. In all other respects the two groups are genotypically alike since heterozygosity in the genomes of the parents may be expected to be randomized among the offspring. Also since the two groups are grown in the same bottle, their environment may be considered identical. If a significant difference in the expression of *R(ci*$^{+}$*)/ci* is observed between the two groups, the difference can be attributed to the Y chromosome.

All Type 1 and Type 2 females were collected within ten-hour intervals to assure virginity, numbered for identification and then classified as to the degree of cubitus vein interruption in the manner described above. Each female was then progeny tested by mating her singly to several *B;Sb/Xa* males in order to determine whether she carried an extra Y chromosome. The presence of the dominant marker, Bar, in the X chromosome of the father served to distinguish between those progeny which were regular for the disjunction of the X chromo-

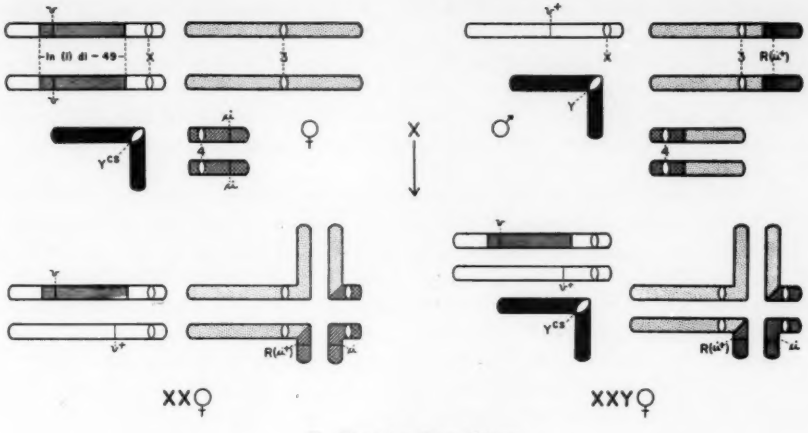

FIGURE 1.—Mating scheme for the production of two groups of daughters which are genotypically equivalent except for the presence or absence of an extra Y chromosome. P_1, *In(1)*dl-49,v^{0l}/*In(1)*dl-49,v^{0l}/Y^{c-s}; *ci ey*^{R}/*ci ey*^{R} ♀ × *R(ci*$^+$*)*/*R(ci*$^+$*)* ♂; F_1 females, Type I. *In(1)* dl-49,v^{0l}/+; *R(ci*$^+$*)*/+; *ci ey*^{R} Type II. *In(1)*dl-49,v^{0l}/+/Y^{c-s}; *R(ci*$^+$*)*/+; *ci ey*^{R}.

somes and those progeny which resulted from nondisjunction of the X's. Regular progeny consisted of *B* females and *B*$^+$ males whereas nondisjunctional progeny consisted of *B*$^+$ females and *B* males. The presence of the dominant marker *Sb* in one of the third chromosomes and of *Xa* in the other third chromosome of the male parent served as a check on the virginity of the female parent. All of the progeny were expected to carry *Sb* or *Xa* and any failure to do so would indicate that the mother was nonvirgin.

It is well known that the frequency of nondisjunctional progeny of females heterozygous for the dl-49 inversion is markedly altered by the addition of a Y chromosome. STURTEVANT and BEADLE (1936) have shown that an XXY female heterozygous for the dl-49 inversion in X produces 45.6 percent secondary nondisjunctional progeny. In contrast, a female heterozygous for dl-49 without a Y produces only 0.1 percent primary nondisjunctional offspring. The difference in the percent of nondisjunctional progeny from females heterozygous for the dl-49 inversion served as the basis for recognizing the presence or absence of a Y chromosome. If the exceptional progeny approached 50 percent, the mother was scored as having a Y. In general no more than two percent of exceptional progeny were observed from mothers scored as XX. The highest number of exceptions observed from such a mother was approximately five percent (8/158). STURTEVANT (MORGAN and STURTEVANT 1944) observed that the presence of a heterozygous inversion in an autosome coupled with a heterozygous inversion in the X will increase the amount of primary nondisjunction and decrease the

amount of secondary nondisjunction of the X chromosomes. A heterozygous translocation coupled with a heterozygous inversion in X appears to have a similar effect. This would explain the deviation from the expected in those cases in which the exceptional progeny arising from primary nondisjunction were too high. This effect was never sufficient to cause any ambiguity in the results and no culture was discarded because of uncertainty as to whether the mother did or did not possess a Y chromosome.

In the case of T(3:4)86D six cultures or one percent were discarded for sterility. All fertile cultures produced haplo-4 flies indicating that both parents were diplo-4. Nonvirginity was absent. The results of this experiment, which are given in Table 1, show that a highly significant difference exists between females with and without a Y chromosome. Furthermore, the effect of the extra Y is partially to suppress the Dubinin effect.

In the case of T(3:4)89E eleven cultures or three percent were discarded for sterility. All fertile cultures produced haplo-4 flies. Nonvirginity was absent. The results of this experiment are given in Table 2. The difference between XX and XXY females is highly significant and, again, the effect of the extra Y is partially to suppress the Dubinin effect.

Comparison of the effect of one and two extra Y chromosomes on R(ci+)/ci: In a second experiment, a comparison of the effect of one and two extra Y chromosomes on the expression T(3:4)86D, $bx^{34e}e^{4}/ci$ was studied. In order to obtain females which carry two extra Y chromosomes, it is necessary to secure

TABLE 1

Distribution according to degree of cubitus interruptus of females of constitution In(1)*dl-49*,v^{0f}/+; *T(3:4)86D*,bx^{34e} e^{4}/+; ci ey^{R} *with and without an extra Y chromosome*

Sex chromosomes	Degree of cubitus interruption*									Totals
	0	1	2	3	4	5	6	7	8	
XX	14	26	64	56	53	26	16	0	0	255
XXY	30	63	82	41	41	21	12	1	0	291
$\chi^2 = 25.89.$		$n = 6$†.		$P < .0003.$						

* For classification, see text. 0 indicates normal for both wings. 8 indicates one eighth or less of the vein for both wings.
† Classes 6 and 7 were combined in calculating χ^2.

TABLE 2

Distribution according to degree of cubitus interruptus of females of constitution In(1)*dl-49*,v^{0f}/+; *T(3:4)89E*,ss bx bxd/+; ci ey^{R} *with and without an extra Y chromosome*

Sex chromosomes	Degree of cubitus interruption*									Totals
	0	1	2	3	4	5	6	7	8	
XX	3	3	9	20	44	55	41	5	0	180
XXY	9	13	25	37	37	31	16	1	0	169
$\chi^2 = 42.20.$		$n = 6$†.		$P < .0001.$						

* See Table 1.
† Classes 6 and 7 were combined in calculating χ^2.

parents, each of which are known to carry one extra Y. This was accomplished by introducing into one of the second chromosomes of each parent the inversions *(2L)Cy*, *Cy* and *(2R)bw^{VDe1}*,*bw^{VDe1}*. The suppression of the dominant brown-variegated phenotype by an extra Y chromosome so as to produce a *Cy* fly with suppressed brown variegation served as the means for identifying the presence of an extra Y chromosome in each parent. Many males and females of the following genotypes

$$\frac{y^2v}{FM3,y^{21d}sc^8dm\ B\ l}\ Y^{c-s};\ \frac{Ins(2L)Cy,\ (2R)bw^{VDe1},Cy\ bw^{VDe1};}{bw}\ \frac{ci\ sv^n}{ci\ sv^n}\ ♀$$

$$\times\ y^2v/Y^{c-s}/Y^{c-s};\ \frac{Ins(2L)Cy,\ (2R)bw^{VDe1},\ Cy\ bw^{VDe1};}{bw}\ \frac{T(3:4)86D,\ bx^{34e}e^4}{+\ ;\ ey^D}\ ♂$$

were pair mated in vials, kept at 25°C for 24 hours and then transferred to bottles. The bottles were kept at 20°C until the offspring hatched.

The non-Bar, non-Curly, translocation-bearing female progeny of such a mating consists of three types which are phenotypically indistinguishable.

These are:

Type I. y^2v/y^2v; *bw/bw* T(3:4)86D/*ci sv^n*

Type II. $y^2v/y^2v/Y^{c-s}$; *bw/bw;* T(3:4)86D/*ci sv^n*

Type III. $y^2v/y^2v/Y^{c-s}/Y^{c-s}$; *bw/bw;* T(3:4)86D/*ci sv^n*

The three types arise in each case from the mating of a single male and female. Consequently, the environmental and genotypic background of each group is equivalent.

Females of Type I, Type II, and Type III were collected within ten-hour intervals to assure virginity, were numbered, graded for degree of cubitus vein interruption and individually mated to *v;bw^{VDe1}/SM1, al^2 Cy sp^2* males. The presence of the yellow^2 (y^2) mutant in both X chromosomes of the female parent and of y^+ in the X chromosome of the male parent served to distinguish between those progeny which arose from the regular disjunction of the X chromosomes and those progeny which arose from the nondisjunction of the X chromosomes. The regular progeny consist of y^+ females and y^2 males, whereas the nondisjunctional progeny consists of y^2 females and y^+ males.

The presence of vermilion (*v*) in both parents and of brown (*bw*) in the female parent served to facilitate the detection of the suppression of variegation. Females which are *v/v;bw^{VDe1}/bw* or males which are *v*/Y;*bw^{VDe1}/bw* have nearly white eyes whereas the addition of an extra Y to either of these genotypes gives a red eye color which is indistinguishable from that of vermilion.

The method of determining the number of Y chromosomes in Type I, Type II, and Type III females is to observe the proportion of *bw^{VDe1}* (non-Curly) progeny of such a female which shows suppression of variegation.

If the female is Type I (XX) then all the *bw^{VDe1}* regular progeny are expected to have unsuppressed brown variegation.

If the female is Type II (XXY) then the *bw^{VDe1}* regular progeny are expected to consist of approximately equal numbers of suppressed and unsuppressed flies.

If the female is Type III (XXYY) then most of the bw^{VDe1} regular progeny are expected to have suppressed brown variegation (STERN 1929).

Eighty-seven females were progeny tested and of these seven or eight percent were sterile. Among the remaining 80 flies, seven flies or nine percent were classified as Type I (XX), 46 or 58 percent were classified as Type II (XXY) and 27 or 34 percent were classified as Type III (XXYY).

Since the females were in all other respects genotypically alike it was possible to compare the effect of no Y, one Y, and two Y chromosomes on their fertility. The average number of non-Curly progeny from an XX mother was 230; from an XXY mother was 146; and from an XXYY mother was 34. Thus fertility in the female appears to vary inversely with hyperploidy for the Y chromosome.

All of the Type I and Type II females produced bw^{VDe1} haplo-4 flies. Four of the Type III females produced no bw^{VDe1} haplo-4 flies. In view of the small total numbers of bw^{VDe1} progeny from XXYY females, the absence of the class is occasionally expected. The number of progeny nondisjunctional for the X's was less than two percent for each type.

The results of this experiment, which are given in Table 3, show that a highly significant difference exists between females with one Y chromosome (Type II) and those with two Y chromosomes (Type III). The effect of two extra Y's is to suppress the Dubinin effect to a greater extent than does one extra Y chromosome. The number of Type I (no extra Y) females was too small to permit a statistical analysis of this group.

Effect of one extra Y chromosome on homozygous ci: Although the addition of one or two extra Y chromosomes partially suppresses the Dubinin effect, it is possible that extra Y's might have a similar effect on the *ci* mutant. At least one mutant, sparkling (L. V. MORGAN) which is unlocated in chromosome 4, is known to behave in this way. XX females and XO males exhibit the sparkling characteristic whereas XXY females and XY males do not. It seems unlikely that a Y chromosome would have a similar effect in the case of *ci* since STERN, MACKNIGHT and KODANI (1946) reported that homozygous *ci* males have a more extreme **ci** phenotype than homozygous *ci* females. Nevertheless, the possibility was tested in the following experiment.

Females of the genotype *In(1)*dl-49,v^{0f}/*In(1)*dl-49,v^{0f}/Y^{c-s};*ci* ey^R/*ci* ey^R were

TABLE 3

Distribution according to degree of cubitus interruptus of females of constitution y^2v/y^2v; bw/bw; *T(3:4)86D*,bx^{34e} e^4/+; ci sv^n *with one or two extra Y chromosomes*

Sex chromosomes	Degree of cubitus interruption*									Totals
	0	1	2	3	4	5	6	7	8	
XXY	1	1	2	1	5	7	28	1	0	46
XXYY	1	2	1	4	7	8	4	0	0	27

$\chi^2 = 16.19$. $n = 3$†. $P < .001$.

* See Table 1.
† Classes 0–3 and classes 6 and 7 were combined in calculating χ^2

mated to males homozygous for *ci gvl bt*. F_1 virgin females whose genotype was *In(1)*dl-49,v^{of}/+; *ci* ey^R/*ci gvl bt* or *In(1)*dl-49,v^{of}/+/Y^{c-s}; *ci* ey^R/*ci gvl bt* were numbered, graded and mated singly to *B; Sb/Xa* males to determine if an extra Y chromosome was present. Six cultures or 2.4 percent were discarded for sterility. Nonvirginity was absent.

The results of this test, which are given in Table 4, show that an extra Y chromosome has no significant effect on the expression of the homozygous *ci* mutant.

DISCUSSION

The results which have been obtained in these experiments show that the phenotype of position effects of *R(ci⁺)* is susceptible to modification by extra Y chromosomes. The degree of modification is greater in the presence of two extra Y chromosomes than in the presence of one extra Y. The homozygous *ci* mutant, on the other hand, is not significantly affected by the addition of an extra Y. This evidence, in conjunction with the euchromatic-heterochromatic type of rearrangement which is known to be involved in these translocations, greatly increases the likelihood that the Dubinin effect is a variegated type position effect.

GOWEN and GAY (1933) discovered that the addition of an extra Y chromosome suppresses the variegation of a euchromatic locus. This suppression has since been confirmed for all variegating euchromatic loci which respond to changes in the number of Y chromosomes and has been generalized into a rule (MORGAN and SCHULTZ 1942). Contrariwise, position effects for rearrangements of the light gene, whose locus lies either within or close to proximal heterochromatin of chromosome 2, are enhanced by an extra Y and suppressed in the XO condition (SCHULTZ 1936). Since light is the only heterochromatic locus for which the effect of different doses of the Y chromosome is definitely known and since the Y effect is in this case in the direction opposite to that observed for euchromatic loci, a second "rule" has emerged which states that variegation of "heterochromatic" loci is enhanced when an extra Y is added and suppressed the XO condition (MORGAN and SCHULTZ 1942).

This "rule" would have much greater validity if the behavior of other "heterochromatic" loci was found to resemble that of light. Actually, with the exception

TABLE 4

Distribution according to degree of cubitus interruptus of females of constitution In(1)*dl-49*,v^{of}/+; ci ey^R/ci gvl bt *with and without an extra Y chromosome*

Sex chromosomes	Degree of cubitus interruption*									Totals
	0	1	2	3	4	5	6	7	8	
XX	0	0	6	15	18	36	30	5	0	110
XXY	0	0	2	9	20	42	38	11	4	126
$\chi^2 = 8.59$.	$n = 4$†.		$P = .075$.							

* See Table 1.
† Classes 2 and 3 and classes 7 and 8 were combined in calculating χ^2.

of *lt* and *ci* little is known concerning position effects of "heterochromatic" loci in *D. melanogaster*. This is due, at least in part, to the facts that such loci are extremely limited in number; that the phenotype of some of these is unsuitable for the study of variegation; and that in at least one case, that of straw, efforts to detect variegation have been unsuccessful (GRELL, unpublished data).

SCHULTZ (MORGAN and SCHULTZ 1942) found that M(2)S10, when heterozygous for the mutant rolled which is located in or close to the proximal heterochromatin of 2R, gave a variable phenotype for rolled. SCHULTZ suggested that if the deficiency of M(2)S10 does not include the locus of rolled, the removal of the heterochromatic block might bring rolled closer to euchromatin and thus cause it to show a position effect. If the behavior of rolled resembles that of light, the addition of an extra Y would be expected to enhance this effect. According to SCHULTZ, such an addition did not affect the manifestation of rolled. Since it is possible that the deficiency for M(2)S10 does include the locus of rolled, this case may not be cogent.

BAKER (1953) reported on the effect of an extra Y chromosome on a *R*(+) of the peach gene which is located within or close to the proximal heterochromatin of chromosome 5 in *D. virilis*. He found that the extra Y had no striking effect on the variegation of this rearrangement although a slight suppression might occur. As BAKER points out, this is not strictly comparable to the light case since the extra Y is added to a Y;5 translocation which is a heterochromatic-heterochromatic rearrangement rather than a heterochromatic-euchromatic rearrangement.

Although position alleles of ci^+ have been studied extensively, the effect of changes in the euchromatic-heterochromatic balance on such position alleles has not been determined with any degree of certainty. Thus KHVOSTOVA (1939) reports that in four cases of *R*(+) of *ci*, the *ci*-index was more extreme when an extra Y was added while in two cases it was less so. She concludes that her data were insufficient and that the problem required further study with more refined methods.

The present experiments were undertaken in the knowledge that the Dubinin effect is difficult to study for a number of reasons. Chief among these are the susceptibility of *ci* position effects to modification by both genetic and environmental factors and the likelihood that fourth chromosome translocation stocks are partially triplo-4. As a preliminary step to these investigations, the stocks which were to be used were cleared of extra fourth chromosomes and were rebalanced so as to prevent the recurrence of this condition. The experiments were planned so that a comparison could be made between populations of otherwise genetically equivalent females which carried different "doses" of the Y chromosome. The populations which were to be compared were reared in the same bottles under carefully controlled temperature conditions. In order to minimize subjective errors, the *ci*-index of the female was determined a generation before her Y constitution was known. Likewise, the Y determination was nearly always made without the knowledge of her *ci* classification.

The results of these experiments show that the Y chromosome partially suppresses the Dubinin effect. A suppression is observed when XXY females are compared with XX females and when XXYY females are compared with XXY females. Although the effect of the XO condition on *R(ci⁺)/ci* has not been studied it is of interest to note that ALTORFER (1952) compared the influence of the XY and XO condition on *R(ci)/ci*. She observed that those *R(ci)* which respond to changes in the Y constitution show an enhancement in the XO condition. This finding is consistent with the result which would be expected on the basis of the present experiments.

Thus extra Y chromosomes modify the Dubinin effect in the same way that they modify the variegation of euchromatic loci and not as they modify position effects of the light gene. Clearly then, on the basis of our present knowledge, no generalization can be made concerning the effect of an extra Y chromosome on the position effect of genes located in or near heterochromatin.

SUMMARY

1. Groups of females which were genotypically equivalent except for the presence or absence of an extra Y chromosome were compared for the expression of *R(ci⁺)/ci*. A highly significant difference was observed between the group with and the group without an extra Y in the cases of the two rearrangements which were studied. The effect of the extra Y in both cases was to partially suppress the Dubinin effect.

2. Two groups of females which were genotypically equivalent except for the presence of one or two Y chromosomes were compared for the expression of *R(ci⁺)/ci*. A highly significant difference was observed between the two groups. The group with two extra Y chromosomes showed a greater suppression of the Dubinin effect.

3. Two groups of females which were genotypically equivalent except for the presence or absence of an extra Y chromosome were compared for the expression of homozygous *ci*. No significant difference was observed between the two groups.

4. The responce of the phenotypic expression of *R(ci⁺)/ci* to changes in the euchromatic-heterochromatic balance greatly strengthens the case for identifying the "Dubinin effect" as a V-type position effect.

5. The Dubinin effect, a position effect for the "heterochromatic" locus *ci*, resembles V-type position effects of euchromatic loci in that it is partially suppressed by extra Y chromosomes. This observation is not consistent with the generalization, based on the case of light, which states that position effects of "heterochromatic" loci are enhanced by the addition of extra Y chromosomes.

6. A comparison of otherwise genotypically equivalent females which carried no extra Y, one extra Y and two extra Y chromosomes showed a highly significant difference in the fertility of the three groups. The fertility of the females varies inversely with hyperploidy for extra Y chromosomes.

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A CYTOLOGICAL STUDY OF w^{vc} CHROMOSOME INSTABILITY IN CLEAVAGE MITOSES OF DROSOPHILA MELANOGASTER[1]

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INSTABILITY of the w^{vc} ring-X chromosome of *Drosophila melanogaster* is attributable to anaphase bridge formation during the cleavage mitoses where loss of the bridge from $w^{vc}/+$ embryos results in gynandromorphs or X0 males and breakage of the bridge results in lethality (HINTON 1955). Rod-w^{vc} chromosomes derived from unstable w^{vc} ring chromosomes also cause lethality, but are not lost during cleavage. If lethality is due to anaphase bridge breakage in both the unstable ring and rod chromosomes, the bridges must arise from sister strand fusion rather than sister strand crossing over. Structural differences between bridges formed by sister strand fusion may account for the difference with respect to gynandromorph production by the two kinds of chromosomes (Figure 1); the ring-w^{vc} double chromatid bridge may be subject to either breakage or loss while the rod-w^{vc} single chromatid bridge may always break (HINTON 1957).

This model of w^{vc} instability is supported by the differences in bridge frequencies for stable and unstable rings observed in larval brain cells (BRAVER and BLOUNT 1949; HINTON 1955) and in secondary spermatocytes (WELSHONS and HINTON 1955). Because of their limited nature and the questionable relevance of observations on these tissues, the results of a cytological study of ring and rod-w^{vc} embryos of the third through eighth cleavages are reported in this paper. These results provide partial confirmation of the anaphase bridge model in that the frequencies of bridges and other mitotic abnormalities are associated with the degree of instability manifested by the ring and rod chromosomes.

METHODS

For convenience of readers not immediately familiar with Drosophila genetics symbolism, it may be appropriate to describe briefly the chromosomes utilized in this study, as follows: two standard rod-X chromosomes, one of normal sequence bearing the mutant yellow (y) and one of the inverted sequence (dl-49) bearing the mutants yellow, white (w) and lozenge (lz^s); two ring-X chromosomes, one of normal sequence (X^{c2}) bearing the mutants crossveinless (cv), vermilion (v) and forked (f) and one of inverted sequence (w^{vc}) having a variegated position effect at the white locus; a rod-w^{vc} chromosome ($w^{vc}.B^s$) derived from the w^{vc} ring and bearing the proximal segment of the Bar of Stone translocation as a hyperploid second arm; finally, two Y chromosomes, one standard, the other

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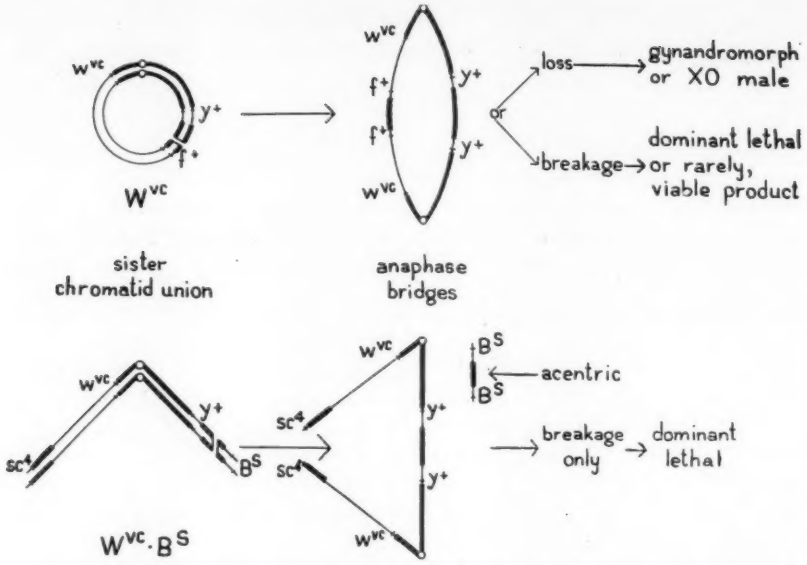

FIGURE 1.—The anaphase bridge model of w^{vc} chromosome instability comparing the consequences of bridge formation in ring and rod chromosomes. The relative proportions of euchromatin (light line) and heterochromatin (heavy line) are shown arbitrarily.

(sc^8·Y) bearing the tip of the X chromosome and including the wild type allele of yellow.

Genetic and cytological observations were made on two sets of crosses. In one set y/y females were crossed to either unstable w^{vc}/sc^8·Y or stable X^{c2}, $cv\ v\ f/sc^8$·Y males. For the second set w^{vc}·B^S/dl-49, $y\ w\ lz^s$ females were crossed to y/Y males, where the w^{vc}·B^S chromosome (Figure 1) of the female was either stable or unstable. Genetic data were collected from these crosses in the usual manner, and eggs deposited by the females on heavily yeasted food chips were collected and prepared by a Feulgen whole mount technique (VON BORSTEL and LINDSLEY 1959).

During preliminary examination, technically good eggs in the third through eighth cleavages were selected for further study; subsequent observations were made from slides coded with respect to stability or instability but not with respect to ring or rod. The cleavage nuclei were classified according to mitotic stage and recorded under either of two groups, prophase-metaphase or anaphase-telophase. The number of prophase-metaphase figures observed is based upon counts (which agreed closely with the geometric increase expected from synchronous mitoses) for the third through sixth cleavage embryos and upon the expected numbers of 64 and 128 for the seventh and eighth cleavage embryos where accurate counts could not be made readily. The anaphase-telophase data include only

those figures classifiable with respect to presence or absence of anaphase bridges, defined as extensive chromatin strands connected with or isolated between the two polar groups of chromosomes of late anaphase or telophase stages. It is possible that use of this definition resulted in the omission of some broken bridges, which may have had the same appearance as chromosome lags often encountered in early anaphase. Because of the compact arrangement of the chromosomes in mitotic figures, the chromosomes involved in bridges could not be identified; for the same reason, it was not possible to distinguish that half of the embryos expected to carry the chromosome under investigation. This latter defect precludes treatment of the data by ordinary statistical tests.

RESULTS AND DISCUSSION

Genetic results: The differences between the stable and unstable ring crosses and between stable and unstable w^{vc}·B^s crosses seen in Table 1 are comparable with those previously reported (HINTON 1955, 1957). The unstable w^{vc} ring cross produced a high frequency of gynandromorphs, X0 males and dominant lethals (= rod ♂ − w^{vc} ♀ − Gy − X0 ♂/rod ♂), while these manifestations of instability were negligible in the stable X^{c2} cross. There were no gynandromorphs and relatively few X0 males produced by either of the two w^{vc}·B^s crosses illustrating the lack of somatic loss of this chromosome. Dominant lethality appeared in both the w^{vc}·B^s crosses; that of the stable cross is primarily attributable to hyperploidy of the B^s arm of the chromosome, and an equal amount of lethality from this source probably exists also in the unstable w^{vc}·B^s cross. The remaining dominant lethality (0.569 − 0.103 = 0.466) is a measure of the total instability of the unstable w^{vc}·B^s chromosome. The total instability of the unstable w^{vc}·B^s chromosome was lower than that of the unstable w^{vc} chromosome. However, this difference is not significant in that the w^{vc}·B^s chromosome was not derived directly from the unstable w^{vc} stock used in this study.

Cytological results: The cytological observations are presented according to cleavage stage in Table 2 for each of the four chromosomes investigated. The

TABLE 1

Genetic results from crosses of y/y *females to ring/*sc^s·*Y males and of* w^{vc}·B^s*/dl-49,* y w lz^s *females to* y/*Y males*

Chromosome	Normal rod males	Normal rod females	w^{vc} females	Gy	XO males	Dominant lethals
Unstable w^{vc}	4519	...	1268	279	455	
Percent	100.0		28.1	16.2		55.7
Stable X^{c2}	2544	...	2533	3	0	
Percent	100.0		99.6	0.1		0.3
Unstable w^{vc}·B^s	260	292	121	0	5	
Percent		100.0	41.4	1.7		56.9
Stable w^{vc}·B^s	945	1341	1195	0	8	
Percent		100.0	89.1	0.6		10.3

TABLE 2

Cytological results from crosses of y/y females to ring/sc^{8}·Y males and of w^{vc}·B^{8}/dl-49, y w lz^{s} females to y/Y males

Chromosome	Cleavage	Number of embryos Observed	Number of embryos Abnormal	Prophase-metaphase Number of figures Observed	Prophase-metaphase Number of figures Abnormal	Anaphase-telophase Number of figures Classifiable	Anaphase-telophase Number of figures With Bridges
Unstable	3	9	6	36	15	0	..
w^{vc}	4	12	4	96	25	0	..
	5	8	6	96	27	19	2
	6	22	10	512	29	100	2
	7	29	13	1088	58	454	25
	8	12	2	768	0	579	4
	Total	92	41	2596	154	1152	33
	Percent	..	44.6	..	5.9	..	2.9
Stable	3	4	1	8	1	6	0
X^{c2}	4	8	1	32	0	27	1
	5	5	0	32	0	23	0
	6	8	1	96	0	99	1
	7	8	5	192	0	213	5
	8	6	0	392	0	177	0
	Total	39	8	752	1	545	7
	Percent	..	20.5	..	0.1	..	1.3
Unstable	3	11	5	40	13	2	0
w^{vc}·B^{8}	4	19	3	128	12	24	0
	5	16	1	240	16	16	0
	6	12	4	288	1	30	3
	7	21	4	1024	18	84	3
	8	31	1	3456	0	181	6
	Total	110	18	5176	60	337	12
	Percent	..	16.4	..	1.2	..	3.6
Stable	3	11	1	40	0	4	1
w^{vc}·B^{8}	4	14	0	96	0	16	0
	5	18	0	240	0	25	0
	6	14	2	320	3	50	1
	7	14	0	896	0	0	..
	8	16	2	1408	0	175	2
	Total	87	5	3000	3	270	4
	Percent	..	5.7	..	0.1	..	1.5

frequency of embryos having one or more nuclear abnormalities was higher in the unstable than in the stable crosses for both the ring and rod chromosomes; this gross consideration does not take into account the fact that there were many more embryos with more than one nuclear abnormality in the unstable than in the stable crosses. It may be noted that the highest frequency of abnormal embryos was less than 50 percent as expected if all the nuclear abnormalities are reflections of instability of the w^{vc} chromosome which should be present in only half the embryos. One might expect the incidence of embryos with nuclear abnormalities to increase exponentially with cleavage stage if the probability of nuclear

abnormality remains constant with cleavage stage; the data, however, suggest no such increase in incidence of abnormal embryos.

The nuclear abnormalities included not only anaphase bridges as previously defined, but a second more frequent class characterized by pycnosis, chromosome fragmentation, increased amounts of chromatin, and tripolar spindles (Figure 2). Nuclei possessing these qualitatively and quantitatively variable characteristics were found adjacent to normal mitotic figures (for a description of the normal mitotic cycle in cleavage nuclei, see SONNENBLICK 1950). The presence of spindles and the oriented arrangement of chromatin in some abnormal figures suggested abortive divisions, which may account for the increased amounts of chromatin. Mitotic derangements similar to those seen in this material have been described previously in Drosophila embryos having excessive polyspermy (HUETTNER 1927), produced by species hybrid females (KAUFMANN 1940), arising from X-rayed gametes (SONNENBLICK 1940), having no X chromosome (POULSON 1940) and treated with ether (CORNMAN 1944). The frequencies of abnormalities for prophase-metaphase mitoses in the stable ring and rod crosses were hardly higher than one might expect in any "normal" material, whereas embryos from both unstable crosses possessed a high abnormality frequency which declined with cleavage stage. No abnormalities were recorded among eighth cleavage prophase-metaphase figures; this fact may be due to including in the data only those nuclei that had completed peripheral migration while those

FIGURE 2.—Camera lucida drawings (ca. 2730 ×) of cleavage nuclei showing pycnosis, chromosome fragmentation, and increased amounts of chromatin.

nuclei having defects remained unrecorded in the yolk mass. Omission of the yolk nuclei was deliberate in that frequent mitotic disturbances have been reported to occur regularly among them (RABINOWITZ 1941).

The data on anaphase bridges suggest no consistent frequency variation with cleavage stage. The bridge frequencies were higher for the unstable than for the stable ring and rod crosses, although the statistical significance of these differences is difficult to assess. The twofold difference in bridge frequency between the X^{c2} and w^{vc} material parallels that found by BRAVER and BLOUNT (1949) in brain smears of X^{c2}/dl-49 and w^{vc}/dl-49 larvae where the bridge frequencies were 12 and 22 percent, respectively. Summing the data from all four crosses, there were 56 bridges observed among 81 embryos having 2306 classifiable late anaphase or telophase figures. If it is assumed that only half of these figures contained a ring or w^{vc}·B^s chromosome and that bridges were limited to this half of the figures, the over-all bridge frequency could be calculated as 56/1153 or 4.8 percent. However, 29 of the bridges were seen in just four embryos having a total of 206 figures (the individual embryo counts were 5/50, 8/24, 10/58 and 6/74 bridges per figures, where the first three cases were seventh cleavage w^{vc} embryos and the fourth was an eighth cleavage w^{vc}·B^s embryo). The remaining 27 bridges were distributed one or two per embryo. This apparent clustering of bridges in particular embryos suggests the occurrence of a bridge breakage fusion cycle of the chromatid type (MCCLINTOCK 1941). In the four embryos cited above, the distribution of figures with and without bridges appeared to be not in sectors as one might expect if the several bridges were related but rather at random. This might be explained in either of three ways: there may be intermingling of the products of normal and bridge anaphases during nuclear proliferation, or some of the breaks may heal rather than fusing, or as SCHWARTZ and MURRAY (1957) have suggested, the fusion forming secondary bridges may be incomplete and weak so that breakage occurs so early in anaphase as to escape cytological detection.

The anaphase bridge model makes two predictions which are supported by the observations. First, bridges formed by ring chromosomes should be double, while bridges formed by rod chromosomes should be single. Of the 16 bridges found in embryos from the w^{vc}·B^s cross, none were visibly double, while 15 of the 40 bridges recorded from the ring crosses were distinctly double; all 15 double bridges were from the unstable w^{vc} cross. As suggested by the drawings of typical bridges (Figure 3), it was frequently difficult to distinguish single and double bridges because of the proximity of the two strands of double bridges. The second prediction supported by the observations is elimination of bridges formed by rings but not of those formed by rods; five of the 40 bridges from the ring crosses were situated between the polar groups of chromosomes so as to suggest their being lost, but none of the 16 w^{vc}·B^s bridges were of this configuration. Other than the differences noted here, there were no recurrent qualitative differences in bridges or other mitotic abnormalities for the different crosses.

The anaphase bridge model also predicts that the bridges produced by sister chromatid union in the w^{vc}·B^s chromosome should be accompanied by an acentric

FIGURE 3.—Camera lucida drawings (ca. 2730 ×) of representative bridge configurations found in cleavage mitoses of embryos from the unstable w^{vc} (top three) and w^{vc}·B^{s} (lower three) crosses.

fragment, but such fragments could be detected in none of the w^{vc}·B^{s} bridge figures seen in this study. This result was preindicated by a similar failure to find acentrics (or bridges) in a preliminary study of w^{vc}·B^{s} larval brain smears (HINTON 1957). The possibility that the acentrics are too small to be seen was tested by examination of anaphase figures in unstable w^{vc}·dl-49 larval brains where the acentric should include at least the entire euchromatic portion of the dl-49 arm of the chromosome. Of 129 anaphase figures observed, four exhibited what may be interpreted either as broken bridges or lagging chromosomes, and five others showed two rod-shaped elements lying isolated between the polar groups of chromosomes (Figure 4). It seems probable that these elements were the pre-

FIGURE 4.—Camera lucida drawings (ca. 2730 ×) of w^{vc}-dl-49 larval brain smear anaphases showing two elements isolated between the polar groups of chromosomes.

dicted acentrics. That there were two such elements per anaphase suggests that the distal chromatid union illustrated in Figure 1 either does not occur or that it persists only briefly, but apparently neither of these alternatives is compatible with production of double dicentrics by unstable ring chromosomes. In this case, one might suppose that the absence of the centromere either prevents the distal sister union or alters its consequences.

An obvious interpretation of these cytological observations in terms of the anaphase bridge model of instability is that bridge breakage results in aberration of the mitotic mechanism which at some later time causes death of the embryo. Because of the statistical inadequacy of the data, their use in attempting to support or deny a causal relation between bridges and other mitotic abnormalities would appear overzealous, especially in view of the lack of information on events occurring between cytologically visible bridges and other mitotic abnormalities. Similarly, the observations offer no direct means of relating the mitotic abnormalities to dominant lethality. Although genetic results (HINTON 1955) have shown that death occurs before larval emergence, none of the embryos, including many in developmental stages later than the eighth cleavage, prepared in this study were obviously necrotic.

SUMMARY

A cytological study was made of Feulgen whole mount preparations of third through eighth cleavage embryos of *Drosophila melanogaster*. These embryos were derived from four crosses involving stable X^{c2}, unstable w^{vc}, and stable and unstable w^{vc}-B^{s} chromosomes. Mitotic abnormalities were scored and included pycnosis, chromosome fragmentation, increased amounts of chromatin and anaphase bridges; the abnormalities were more frequent among embryos from the unstable than from the stable ring and rod chromosome crosses. These results and

other observations were interpreted in terms of the anaphase bridge model of w^{vc} chromosome instability. Evidence was found which suggests the occurrence of a chromatid type breakage fusion bridge cycle in this material.

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RADIOLOGICAL STUDY OF WHEAT MONOSOMICS. I. DIFFERENTIAL SENSITIVITY OF MONOSOMIC X AND THE DISOMIC TO X-IRRADIATION[1]

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MANY papers have been published on differential radiosensitivity due to polyploidy (see summary by SWAMINATHAN 1957) and sex (GLASS 1940; KAWAHARA 1957). However, no systematic method of relating radiosensitivity to specific chromosome number has been devised.

Aneuploid series, such as have been completed in Datura, Oenothera, maize, tomato, tobacco or wheat, facilitate studies relating change in radiosensitivity to the addition or removal of a specific chromosome. Differences in radiosensitivities between the aneuploids and disomics must be, directly and/or indirectly, attributed to the chromosome that varies in dosage in those strains.

The experiment reported here was designed to study the effects that removing a specific chromosome had on the radiosensitivity of the wheat plant subjected to a wide range of X-ray dosages. Such a study should reveal comparative radiosensitivities for specific chromosomes.

MATERIALS AND METHODS

Monosomic and disomic strains of *Triticum aestivum* var. Chinese Spring were used throughout the experiment. Monosomic X of the 21 monosomics available was employed for the experiment. Source of the monosomics was the progeny of self-pollinated monosomic X. The progenies, consequently, were a mixture of disomics, monosomics, and some nullisomic X's. Disomics were obtained from progeny of selfed monosomics to eliminate genetic heterogeneity of the experimental strains.

The seed from the disomics was produced in the greenhouse and the seed from monosomic X in the field. Dormant seeds were treated with X-rays. A G.E. Maxitian X-ray machine, operated at a distance of 35 cm, at 250 KV, and 30 MA, with an aluminum filter 1 mm thick was used. The X-ray dosage per minute was 655r.

Seeds were planted in flats about two weeks after irradiation, and seedlings,

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when three weeks old, were transplanted into six-inch pots. All the plants were grown in a greenhouse.

The experimental design was a split plot, randomized block design with three replications and nine X-ray dosages, i.e., 0, 5, 10, 20, 30, 35, 40, 50, and 60 Kr, for units; and two genetic strains, i.e., disomics and the selfed progeny of monosomic X for subunits. Fifty seeds of each treatment were planted per replication. Data from binomial distribution were transformed to angle prior to analyses. Mainly, analyses of variance and covariance were applied. Variance of the genetic strains was attributable to a difference of general vigor between monosomics and disomics, and partly to differential radiosensitivity of these strains. Variance due to interaction between X-ray dosages and genetic strains was attributed only to the differential radiosensitivity of the two strains. For this reason, the differential radiosensitivity of the monosomics was statistically defined by the interaction between the genetic strains and X-ray dosages.

Radiosensitivity of monosomic X for a specific X-ray dosage was estimated by the following formula:

$$x = \frac{A - (1 - p)\,B}{p}$$

where,

A = X-ray damage to a mixture of monosomic X and disomics.
B = X-ray damage to disomics.
p = proportion of monosomic X in the mixture.
x = estimated damage to monosomic X.

Effect of nullisomic X present in the mixture was neglected, because of its low frequency, estimated as 0.9 percent by SEARS (1954). The proportion of monosomic X in the mixture was previously estimated by the authors (1959).

EXPERIMENTAL RESULTS

A marked difference of seed size occurred between the disomics and the mixture of monosomic X, probably because one was grown in the greenhouse and the other in the field. Analysis of variance indicated that there was no significant difference of seed weights for X-ray dosages, nor for the interaction between the genetic strains and X-ray dosages.

Differential effect of X-rays on the percentage survival

The killing effect of X-rays on the disomics and mixture of monosomic X was recorded at three stages of development; when the first, second, and fourth leaves reached maximum growth. The stages occurred about one, two, and three weeks after sowing, respectively.

The general appearance of the second leaf stage of a demonstration plot is shown in Figure 1. There were no differences in appearance between disomics and the mixture of monosomic X at the lower dosages of X-rays and the control, but remarkable differences occurred following dosages of 30, 35, and 40 Kr.

The actual percentage survival of the disomics and the progeny of monosomic X, and the estimation for monosomic X are given in Table 1.

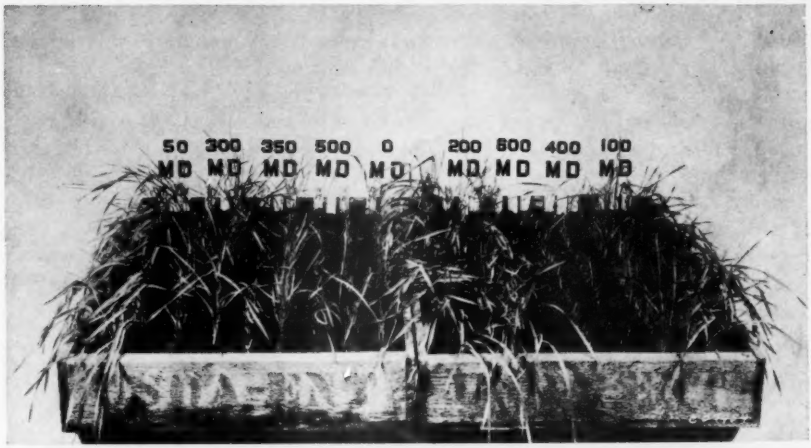

FIGURE 1.—General appearance of a demonstration plot at the second leaf stage, showing differential radiosensitivities of disomics and monsomic X. D: Disomic population. M: Progeny of self-pollinated monosomic X. Number indicates X-ray dosages given in treatment of dormant seeds (in 100r units).

TABLE 1

X-ray effect on the percentage survival of disomics and progeny of monosomic X (mixture), and the estimation of that for monosomic X at the first, second and fourth leaf stages

Developmental stages	First leaf stage			Second leaf stage			Fourth leaf stage		
X-ray dosages (Kr)	Disomics*	Mixture	Estimation for mono. X	Disomics	Mixture	Estimation for mono. X	Disomics	Mixture	Estimation for mono. X
Check	99.3	97.3	95.8	98.7	96.0	94.0	99.3	96.7	94.7
5	98.7	97.3	96.2	98.7	97.3	96.2	98.7	97.3	96.2
10	97.3	98.0	98.5	96.7	97.3	97.8	96.7	98.0	99.0
20	99.3	99.3	99.3	99.3	100.0	100.5	99.3	100.0	100.5
30	98.7	95.3	92.7	98.0	88.0	80.5	98.0	86.0	76.9
35	98.7	90.7	84.7	99.3	62.0	33.9	95.3	49.3	14.6
40	100.0	86.7	76.7	90.0	16.7	—38.6	75.3	7.3	—44.0
50	98.0	66.0	41.9	16.7	4.0	— 5.6	4.7	2.0	0.0
60	96.0	64.7	41.1	8.0	3.3	— 0.2	6.7	2.7	— 0.3

* Values given in percentage.

Statistical analysis of the data indicated that the effects of X-ray, the genetic strains, and their interaction were all very highly significant at all three stages. For the first leaf stage, a slight increase of the percentage survival was noticed with moderate dosages of X-rays in both disomic and mixed populations. A similar observation was reported by WOODWARD *et al.* (1955) in Neurospora. A killing effect of irradiation was not evident in the disomics at any of the X-ray dosages.

However, a decrease in the percentage survival of monosomic X became clear at 30 Kr, and survival decreased gradually at higher X-ray dosages. Survival of this population was not depressed further with X-ray dosages above 50 Kr.

The effects of X-irradiation were more evident at the two leaf stage than at the one leaf stage. At the two leaf stage, the disomics maintained a threshold of resistance to X-rays up to 35 Kr. The killing effect became clear with 40 Kr but was still slight; it increased markedly with an additional dose of 10 Kr. On the other hand, a clear killing effect was observed in the mixture at the dosage of 30 Kr. Clear differences in radiosensitivity between disomics and monsomic X were evident at 30 Kr, were increased at 35 and 40 Kr, and the differences were somewhat reduced at dosages higher than 40 Kr. X-ray dosages inducing 50 percent killing to the populations of the disomics, mixture, and monosomic X were estimated at about 45, 36, and 33 Kr, respectively.

Generally, at the fourth leaf stage there were no marked differences in the killing effect from that of the second leaf stage except that the percentage survival at the fourth leaf stage was less in all cases than in the second leaf stage. X-ray dosages necessary to induce 50 percent killing in the populations of the disomics, mixture, and monosomic X at the fourth leaf stage were estimated at about 44, 35, and 32 Kr, respectively.

Differential effect of X-rays on the seedling height

The effect of X-rays on plant heights was thoroughly analyzed only on the data of the first leaf stage. The heights of plants were measured for all individuals that survived. The data were converted to the average height of seedlings per seed sown to facilitate a statistical analysis. To separate the differential radiosensitivity due to the difference in seed weights, a covariance was worked out on the seed weights and seedling height.

The very highly significant difference in plant heights between the irradiated disomics and the mixture found in the analysis of variance may be attributed to differences in seed weights. On the other hand, differential radiosensitivity between the disomics and the mixture could not be explained on the basis of weight differences.

The X-ray effect on the seedling height of monosomic X was estimated from the average heights of the disomics and mixture, adjusted for seed weights, using the same formula previously described. The seedling heights of the X-rayed disomics and mixture, and the estimation for monosomic X are shown in Table 2.

The disomic population retained its threshold to X-ray injury, at least up to 5 Kr, but monosomic X did not show a clear threshold effect in this experiment. In the range of 5 to 20 Kr, depression of the first leaf development by X-rays was rather slow in the disomics compared with the sharp depression in monosomic X. X-ray dosages from 20 to 35 Kr showed almost the same effects on both, but monosomic X was slightly more sensitive than the disomics. X-ray injury of monosomic X reached a maximum at a dosage of 35 Kr. Rates of depression of plant height in disomics were reduced with dosages over 40 Kr, approaching maximum injury at 60 Kr. X-ray dosages necessary to induce 50 percent reduction of

TABLE 2

X-ray effect on seedling heights per seed sown of the disomics and the mixture, and the estimation of that for monosomic X at the first leaf stage, based on seed weight

X-ray dosages (Kr)	Disomics*	Mixture	Estimation for mono. X
Check	126.1	122.3	119.5
5	125.5	116.0	108.8
10	121.0	111.8	104.9
20	111.3	87.0	68.7
30	71.3	41.0	18.1
35	54.4	25.4	3.5
40	32.7	15.6	2.7
50	18.3	11.2	5.8
60	12.5	10.6	9.2

* Values represent height in mm.

seedling height per seed sown at this stage were estimated as 32, 26, and 22 Kr in the disomics, mixture and monosomic X, respectively.

Differential mutation rates induced by X-rays

Type of mutant obtained: A list of the mutants obtained, their frequencies, and descriptions follows:

Types of mutants obtained	No. of mutants	Description of the mutant characteristics
Speltoid	26	Long, thin and lax spike
Awned	29	Long or short awn
Bilateral mosaic of awn	1	One side of spike awned, the other side awnless
Extra-spikelet	4	Extra spikelets inserted between the regular ones
Double head	3	Two heads on one spike
Double stem	1	Two spikes on one culm
Compound spikelet	1	Florets within a spikelet change to complete spikelets
Chlorophyll mutant	1	One albino streak in each leaf

Pattern of the occurrence of mutations: MacKey (1954) noticed the occurrence of speltoid mutations in chimeras. Data were recorded from plants with two or more heads to check on the mutational response of individual spikes when one of them showed a mutation in order to clarify a pattern of occurrence. The information is shown in Table 3.

The incidence of mutations in each spike order was studied for a range of X-ray dosages from 20 to 60 Kr. The frequency of mutants is summarized in Table 4. Information for the plant, D 23–27 (Table 3), was excluded from estimating mutation rates as this mutation probably originated by mechanisms other than X-irradiation of the seed.

TABLE 3

Segregation of mutant characteristics within a plant

Code no. of plants	X-ray dosages (Kr)	Mutant characteristics	No. of spikes	Order of spikes*				
				1	2	3	4	5
M 21–23	30	Double head	2	—	+	..	..	..
M 38–5	30	Double head	2	+	—	..	..	..
D 23–27	20	Speltoid	3	+	+	+	..	..
M 34–9	20	Speltoid	2	—	+	..	..	..
D 21–27	30	Speltoid	2	—	+	..	..	..
M 38–27	30	Speltoid	2	+	—	..	..	..
M 38–28	30	Speltoid	2	+	—	..	..	..
M 38–34	30	Speltoid	3	—	+	+	..	..
D 14–31	35	Speltoid	3	+	—	—	..	..
M 37–5	35	Speltoid	3	+	—	+	..	..
M 37–18	35	Speltoid	3	—	+	—	..	..
D 18–3	40	Speltoid	3	+	—	—	..	..
D 18–9	40	Speltoid	4	—	+	—	—	..
D 26–1	40	Speltoid	3	+	—	—	..	..
D 26–2	40	Speltoid	2	+	—	..	..	..
D 32–33	40	Speltoid	2	+	+	..	..	..
M 11–1	60	Speltoid	2	+	—	..	..	..
M 13–30	20	Awned	3	+	—	+	..	..
D 38–34	30	Awned	2	+	—	..	..	..
M 15–8	30	Awned	2	—	+	..	..	..
M 15–24	30	Awned	2	+	+	..	..	..
M 38–13	30	Awned	2	—	+	..	..	..
M 38–25	30	Awned	2	+	—	..	..	..
D 14–3	35	Awned	2	+	—	..	..	..
D 14–11	35	Awned	2	—	+	..	..	..
M 14–11	35	Awned	2	+	—	..	..	..
M 14–15	35	Awned	3	—	+	—	..	..
M 37–11	35	Awned	3	+	—	—	..	..
M 37–18	35	Awned	3	+	—	—	..	..
D 18–1	40	Awned	5	—	—	+	—	+
D 18–7	40	Awned	3	+	—	+	..	..
D 18–18	40	Awned	2	+	—	..	..	..
D 32–11	40	Awned	4	+	—	—	—	..

* + mutant spike
— normal spike.

TABLE 4

Incidence of mutations in various spike orders for a range of X-irradiation from 20 to 60 Kr

Spike order	1st	2nd	3rd and later
No. of spikes tested	933	323	187
No. of mutant spikes	44	12	6
Incidence of mutations (percent)	4.7	3.7	3.2

The incidence of mutations was homogeneous through three spike orders. Coincidence of mutation of the second spike with that of the first one was 0.62 percent compared with an expected frequency of coincidence of 0.17 percent. This difference was not significant. On the other hand, coincidence of mutation in the third or later spikes with that of the earlier one was 2.67 percent compared with an expected 0.27 percent, a difference which was significant. These facts suggest that mutations in the first and second spikes probably occurred independently of each other but those in the third or later spikes were somewhat incidental with the mutation in the earlier spikes. On this account, the mutation rates in the following section were calculated from the number of mutant heads appearing in the first and/or second spikes, divided by the total number of the first and second spikes, and excluding data on the third and later spikes.

Differential frequencies of speltoid and awned mutations: Speltoid and awned mutants were the commonest types obtained in the experiment. The frequencies of these mutations in the disomics and mixture of monosomic X are summarized in Table 5.

To compare mutation rates of these characteristics in the disomics and mixture of monosomic X, data corresponding to 20, 30, and 35 Kr of X-ray dosages were combined. Data for X-ray dosages of 10 Kr or less, and 40 Kr or more were excluded in estimating the mutation rates, because the rates were too low to be

TABLE 5

Frequencies of speltoid and awned mutants in the disomic and mixture of monosomic X

X-ray dosages (Kr)	Genetic strains*	No. of plants tested	No. of heads tested	Speltoidy		Awnedness	
				No.	Percent	No.	Percent
Check	D	30	55	0	0.0	0	0.0
Check	M	30	56	0	0.0	0	0.0
5	D	30	54	0	0.0	0	0.0
5	M	30	52	0	0.0	0	0.0
10	D	30	47	0	0.0	0	0.0
10	M	30	40	1	2.5	0	0.0
20	D	149	187	1	0.5	0	0.0
20	M	150	217	3	1.4	6	2.8
30	D	147	195	1	0.5	2	1.0
30	M	129	192	4	2.1	9	4.7
35	D	143	191	5	2.6	3	1.5
35	M	74	94	2	2.1	5	5.3
40 or more	D	130	169	8	4.7	5	2.9
40 or more	M	18	19	1	5.3	0	0.0
20 to 35	D	439	573	7	1.22	5	0.87
20 to 35	M	353	503	9	1.79	20	3.98

* D = Disomics.
M = Mixture.

included in the former, and too few plants of the monosomics survived in the latter.

The mutation rates of the two characteristics after exposure to 20 to 35 Kr of X-irradiation are seen in the last two rows of Table 5. Mutation rates of the speltoid in the disomics and mixture of monosomic X were not significantly different. On the other hand, the mutation rate to awnedness was much higher in the mixture of monosomic X than in the disomics, the χ^2 test indicating a very highly significant difference.

DISCUSSION

Monosomic irradiation as a means of studying functional importance of the individual chromosomes: Genetic lethal effects of X-rays were classified into dominant and recessive ones by ATWOOD *et al.* (1956). Experimental results obtained by STADLER (1929) in oats and wheat; SMITH (1946) in barley, rye and maize; SAX and SWANSON (1941) in Tradescantia; LUCKE and SARACHEK (1953) in yeast; and CLARK (1957) in Habrobracon, all indicated that polyploids were less sensitive to X-rays than diploids or haploids, suggesting that a majority of the lethal mutations that occur are recessive. BEAM (1955) estimated a relative frequency of dominant and recessive lethal mutations at about 15 to one in yeast.

In the monosomics, a single dose of a specific chromosome increases the chance of expressing recessive mutations while it decreases the chances for dominant mutation expression. On the assumption that the former effect is larger than the latter, the monosomics are expected to be more sensitive than disomics to X-irradiation.

In the actual estimation of the differential radiosensitivity of the monosomics, effects of certain factors not associated with genetic difference can be considered. The first one is sampling error. Such a nondifferential effect associated with sampling itself was used as a standard in a statistical evaluation of the differential radiosensitivity. The second group of factors could have a differential effect on two genetic strains. It was reported by many workers (SWAMINATHAN 1957) that age, moisture or size of seeds, wave length of X-rays, or oxygen content of air has an effect on radiosensitivity. The monosomic and disomic seeds used in the experiment were 9 and 12 months old, respectively, and were stored in the same place 9 months before treatment. Of course, the conditions of irradiation were the same for both strains. Seed size did have some effect on the radiosensitivities, so covariance of the seed weight and seedling height per seed sown was calculated. Analyses showed that differential sensitivity of the mixture of monosomic X from disomics was not due to the difference of their seed weights. The third factor considered was the frequency of monosomic X in the mixture. The higher the actual frequency of the monosomics over the expected number, the more the sensitivity of the monosomics is overestimated.

The frequency of monosomic X, from a study of 55 plants (TSUNEWAKI and HEYNE 1959), averaged 57 percent. The 95 percent fiducial limits of the frequency of this monosomic were 78 and 35 percent. SEARS (1954) estimated that

the expected frequency of monosomics was about 75 percent. Estimated sensitivity of monosomic X to a dose of 40 Kr was unusual in most cases. A possible reason may be that the frequency of monosomic X was greater than 57 percent. The percentage survival of the monosomics at a dose of 40 Kr at the second leaf stage was calculated as −36.6 percent using 57 percent as the expected occurrence of the monosomic. A more realistic figure of −4.0 percent was estimated when the frequency of monosomic X was considered to be 78 percent.

These considerations lead to the conclusion that the differential radiosensitivity observed was due to different dosages of chromosome X in the disomics and monosomic X. X-ray dosages necessary to induce 50 percent killing of disomics and monosomic X were estimated as 45 and 33 Kr at the second leaf stage, and 44 and 32 Kr at the fourth leaf stage. Also, X-ray dosages inducing a 50 percent reduction of the average seedling height per seed sown were estimated at 32 Kr for disomics and 22 Kr for monosomic X at the first leaf stage. Differences of the dosages are 12, 12 and 10 Kr in the three different measurements, which are rather constant. This suggests that the difference of LD_{50} between various monosomics and disomics can be used as a numerical index of the functional importance of the corresponding chromosomes.

Monosomics are vigorous enough to facilitate a population study only in polyploid species possessing sufficient duplicate chromosomes originating from the ancestral chromosome. GAINES (1927), and CLAUSEN and CAMERON (1950) pointed out the presence of duplicate factors in hexaploid wheat and oats, and in tetraploid tobacco. SEARS (1952) showed that the three homoeologous chromosomes of common wheat, which supposedly originated from the same ancestral chromosome, have very similar functions, each of which compensates for the functions of the others. RILEY *et al.* (1958) showed that nonpairing of homoeologous chromosomes in polyploid wheats was due simply to a specific gene on chromosome V, as identified by OKAMOTO (1957), rather than nonhomology of their genetic structure. Such duplication of genes was thought to be a reason for the tolerance of polyploids to irradiation.

However, there is other evidence which points to functional divergence of homoeologous chromosomes. STEPHENS (1951) showed that functions of *Cl* and *R* loci were differentiated between subgenomes in tetraploid cotton. SEARS (1958) also found in the study of functional compensation that some chromosomes belonging to the same homoeologous groups did not show typical compensating effects with each other. Gene analyses of hexaploid wheats using monosomic and nullisomic series provided information on both functional similarity and dissimilarity of the homoeologous chromosomes for growth habit (SEARS 1954), chlorophyll production (SEARS 1957), stem solidness (LARSON 1952) and awnedness (SEARS 1954 and others).

Differential radiosensitivity of monosomics in wheat may depend on the degree of differentiation of the monosomic chromosome from the other homoeologues as the dosage of the differentiated genes located on the specific chromosome changes from double to single, but that of nondifferentiating genes from quadruple to

triple or six-fold to five-fold by removing the chromosome in the monosomics. Thus, the differential radiosensitivity of the monosomics could be an index of differentiated functions of the chromosome, including some effect of its common functions to the other homoeologues.

Monosomic irradiation as a means of studying the functional importance of individual chromosomes is rather restricted in its use since complete series of monosomics are available only in tetraploid tobacco and hexaploid wheat. However, radiological study of a trisomic series may provide favorable materials for the same purpose in other species.

Monosomic irradiation as a means of screening mutants: DAVIS (1950) and others reported a method of screening biochemical mutants by using the differential sensitivity to penicillin of dividing and nondividing cells. WOODWARD *et al.* (1954) devised another type of screening with methods based on differential germination of wild and mutant conidia of Neurospora in minimum media. These are now widely used in screening auxotrophic mutants in bacteria and fungi. However, no such method has been developed to screen mutants in higher organisms. The irradiation of monosomics reported here provides a way of screening mutants based on the differential dosage of certain genes in the monosomics.

Genes carried on a monosomic chromosome are in single dose and all genes located on the other chromosomes are in double dose. It is assumed here that a gene on a specific chromosome mutates to the dominant alleles with a frequency p. or to the recessive alleles with a frequency of q, for a certain X-ray dosage. Theoretically, the frequency of dominant mutants appearing in the X_1 generation must be p in the critical monosomics and $2p-p^2$ in all the other monosomics as well as in the disomics. As p is generally much smaller than one, the frequency of dominant mutations in the critical monosomics is expected to be about one half of that in disomics and the other monosomics. On the other hand, the frequency of recessive mutants is q in the critical monosomics and q^2 in the others. The expression of the recessive mutations, therefore, should be $\frac{1}{q}$ times higher in the critical monosomics than in other populations. Theoretical efficiency of screening recessive mutants for duplicated genes is given in Table 6. For example, the efficiency of screening a mutation is about 100 times greater in a critical monosomic than in other populations when the mutation rate is one percent and a single duplicate gene pair is involved.

The theoretical considerations above are partially supported by the data on speltoid and awned mutants obtained in the experiment. The genetic basis of these mutations is well known in Chinese Spring (SEARS 1954). The speltoid mutation is due to a deficiency of the Q gene carried on chromosome IX and is dominant. The awned mutation is due to either a duplication of the gene on chromosome II, XIII, or XX; or a deficiency of the gene located on chromosome VIII or X, all of which are recessive. As previously described, the frequencies of speltoid mutants were 1.22 percent in the disomics and 1.79 percent in the mixture of monosomic X for a range of X-ray dosages from 20 to 35 Kr. The difference was not significant,

TABLE 6

Relative efficiency of screening recessive mutants of duplicated genes by irradiating monosomics in comparison with irradiating disomics

q \ n	1	2	3	n
1×10^{-4}	10,000	5,000.5	3,334.0	$1-(1-10^{-4})(1-10^{-8})^{n-1}$
1×10^{-3}	1,000	500.5	334.0	$1-(1-10^{-3})(1-10^{-6})^{n-1}$
1×10^{-2}	100	50.5	34.0	$1-(1-10^{-2})(1-10^{-4})^{n-1}$
1×10^{-1}	10	5.5	4.0	$1-(1-10^{-1})(1-10^{-2})^{n-1}$
q	$\frac{1}{q}$	$\frac{1+q-q^2}{2q-q^3}$	$\frac{1+2q-2q^2-q^3+q^4}{3q-3q^3+q^5}$	$1-(1-q)(1-q^2)^{n-1}$

n = number of duplications of a gene pair.
q = average frequency of the recessive mutation.

as was expected, as the dosage of the Q gene is the same in both populations. On the contrary, frequencies of the awned mutants were 0.87 percent in disomics and 3.98 percent in the mixture of monosomic X to the same range of X-ray dosages. The difference was very highly significant, which was expected also because one of the five genes responsible for awn expression is located on chromosome X. The significant difference probably was not due to the presence of awned nullisomic X in the mixture of monosomic X. First of all, nullisomic X is undoubtedly much more sensitive to irradiation than the monosomics, so that most of the nullisomics probably were killed with an X-ray dosage of 20 Kr or more. Actually, all awned mutants with more than one spike showed segregation of spikes for awnedness. Second, the frequency of nullisomic X was only about 0.9 percent, so the awned nullisomics could have contributed only one fourth of the total frequency of the mutants in the mixture, even if there was no killing of the nullisomics.

The irradiation of monosomics as a means of screening mutants has several important aspects. First, it is an efficient means of screening recessive mutants. One can identify a chromosome that carries a mutant locus by a discrepancy of the mutation rates observed in the critical monosomics and the other populations, independently of the nature of the mutations. Second, a complete series of monosomics is available only in a limited number of polyploid species and a high degree of gene duplication in such polyploids lowers the efficiency of the screening. Finally, the irradiation of dormant seeds of monosomics as reported here cannot be directly applicable to seedling characteristics because differentiation has taken place to a certain extent before irradiation. This is evident from the fact that the first and second spikes within a plant exhibited an independent response to a mutation with respect to each other. However, a modified method of irradiation, such as irradiation of the ovaries of monosomic plants just after fertilization or treatment of an entire population of monosomics in the flowering stage in a radiation field, may provide a useful way to screen mutants even for seedling characteristics.

SUMMARY

A new approach to radiation genetics is described. This study involves the analyses of differential sensitivity of a series of aneuploids to X-irradiation. Differential dosage of a specific chromosome may be reflected in differential radiosensitivity of the aneuploid and the disomics. At the same time, differential dosage of a group of genes located on the specific chromosome may result in a differential frequency of specific mutants in the X_1 generation.

Our approach to this problem involved a study of radiation responses of monosomic X and disomics of *Triticum aestivum* var. Chinese Spring after irradiation of dormant seeds with a wide range of X-ray dosages. Seeds obtained from self-pollinated monosomic X were used as a source of monosomic seeds assumed to be a mixture of disomics, monosomic and nullisomic X with an approximate proportion of 42 percent, 57 percent, and 1 percent, respectively. Consequently, radiosensitivity of monosomic X could be estimated from the radiosensitivities of disomics and the mixture of monosomic X, and its frequency in the mixture, neglecting the effect of the nullisomics present.

Differential radiosensitivity of the monosomics was statistically defined by the interaction between the genetic strains and X-ray dosages. Covariance analysis indicated that the differential radiosensitivity was attributable very little, if any, to the difference of the seed weights.

Monosomic X was much more sensitive than disomics to moderate dosages of X-rays. The X-ray dosages necessary to induce 50 percent killing were 45 and 33 Kr at the second leaf stage, and 44 and 32 Kr at the fourth leaf stage, for disomics and monosomic X, respectively. The dosages inducing 50 percent reduction of seedling height per seed sown were 32 Kr for disomics and 22 Kr for monosomic X at the first leaf stage. The differences were 12, 12, and 10 Kr, which were rather constant, suggesting that such a difference of LD_{50} can be used as a numerical index of relative radiosensitivity of individual chromosomes.

Mutational responses of the X_1 generation of monosomic X and disomics were analyzed in detail for the speltoid and awned mutations over a range of 20 to 35 Kr of X-rays. It was clear that mutations occurred independently in the first and second spikes but were similar in the later spikes, so that frequencies of the mutants have to be calculated on the basis of the total number of the first and second spikes tested. The frequencies of the speltoid mutants were 1.2 percent in the disomics and 1.8 percent in the mixture of monosomic X. This difference was not significant, as was expected because the speltoid factor is located on chromosome IX and both disomics and monosomic X have the same chromosome IX gene dosage. On the contrary, frequencies of the awned mutants, of which one factor is located on chromosome X, were 0.9 percent in disomics and 4.0 percent in the mixture. This difference was very highly significant. These data suggest that irradiation of the monosomics is an efficient way of screening recessive mutants in the X_1 generation, even for highly duplicated genes.

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RADIOLOGICAL STUDY OF WHEAT MONOSOMICS. II. DIFFERENTIAL SENSITIVITY OF 16 MONOSOMICS AND THE DISOMIC TO A SINGLE DOSAGE OF X-IRRADIATION[1]

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THE radiological study of monosomic X of *Triticum aestivum* reported by TSUNEWAKI and HEYNE (1959b) pointed out the merits of irradiating seeds of monosomics as an approach to the study of the functional importance of individual chromosomes as well as a method for screening mutations. In this paper, the results of irradiating 16 monosomics and the disomic are reported.

The dosage of a chromosome is different in a specific monosomic than in the disomic or any other monosomic. When the chromosome as a whole has a specific function for a certain developmental stage, a radiological effect should express itself more intensely in a specific monosomic than in other monosomics at that developmental stage. Therefore, a comparative study of radiological responses of various monosomics should provide an experimental approach to the study of specific functions of individual chromosomes at various developmental stages. A moderate dosage of irradiation appears to be favorable for this purpose, as a drastic change of any developmental stage may upset the entire developmental process.

MATERIALS AND METHODS

Radiosensitivities of 16 of the 21 monosomics available in *Triticum aestivum* var. Chinese Spring, were comparatively studied by irradiating dormant seeds with 14 Kr units of X-rays. The X-ray machine was operated at a distance of 15 cm, without a filter, at 80 KV and 18 MA.

The seeds were sown in greenhouse flats, and allowed to grow for four weeks before being transplanted to the field.

The experimental design used was a split plot in a randomized block design with five replications and 17 genetic strains, i.e., the disomic and 16 monosomics, for units and two treatments, namely irradiation and check, for subunits. One hundred seeds were planted for each subunit, so 500 seeds were subjected to the same treatment.

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Different radiological responses of the plant were studied at three stages of development, i.e., germination percentage, seedling heights at the first leaf stage, and number of tillers produced.

The results were analyzed by analysis of variance and covariance in which the effect of interaction between the genetic strains and treatments was used as a criterion for the differential radiosensitivities of the monosomics.

The monosomics were derived from progenies of self-pollinated monosomics, whose chromosome number had been checked cytologically. The progenies, consequently, were mixtures of di-, mono- and nullisomics. Frequencies of the nulli- and monosomics in the mixture were estimated by SEARS (1954) and the authors (TSUNEWAKI and HEYNE 1959a) for each chromosome. Radiosensitivity of a "pure" monosomic population was estimated from the formula presented in a previous paper (TSUNEWAKI and HEYNE 1959b). The effect of nullisomics present in the mixtures was neglected in the estimation because of their low frequencies.

EXPERIMENTAL RESULTS

A marked difference in seed sizes occurred among the 17 strains as the disomic seed came from the greenhouse and the monosomic was grown in the field. Therefore, 100-kernel weights were recorded for each plot. The effects of the irradiation on germination percentage, seedling height at the first leaf stage, and numbers of tillers produced at the tillering stage, along with seed weight, are summarized in Table 1.

Differences of seed weights of the 17 strains were very highly significant. Least significant difference at the five percent level indicated that the disomic was the heaviest, and progenies of monosomics VI, II, and XVII were the next heaviest class. Progeny of monosomic IX was the lightest and those of monosomics XIII, XI, X, and VIII were the next lightest class. The remaining eight monosomic progenies belonged to the medium class.

The differential effect of X-rays on germination percentage of the 17 strains was significant. The percentage germination of the progeny of monosomic XIII was significantly decreased and that of monosomic II was significantly increased by irradiation. The differential radiosensitivity studied on seedling height was highly significant. The progenies of monosomics III, XV, X and XVI seemed to be more sensitive and the disomic was more resistant than the average. The differential effect of X-rays on tillering was significant. The progenies of monosomic IV and the disomic were not reduced in comparison with a noticeable reduction of the number of tillers in the other progenies.

Correlations among the relative radiosensitivities of the 17 strains at the three different developmental stages were studied, including differences of seed weights. The results are shown in Table 2.

The correlations between seed weight and radiosensitivity observed in all three stages of development were not significant. Covariance between 100-kernel weights and the seedling heights indicated that the differential radiosensitivity

observed for the seedling heights was not accounted for by differences in seed weight. However, consideration of different seed sizes rather improved the level of significance of the differential radiosensitivity through reduction of error variance attributed to seed weight. The result of the covariance together with those of the correlation study suggest that the differences in seed weight were not a main factor in the differential radiosensitivities of the monosomics.

No significant correlations were found between differential radiosensitivities observed in germination and the two later stages of development studied. How-

TABLE 1

100-kernel weights and X-ray effects on percentage germination, seedling height at the first leaf stage, and number of tillers at the tillering stage of the disomic and selfed progenies of 16 monosomics

Genetic strains; progenies of	100-kernel weight (gm)	Germination decrease*	Seedling height reduced*	No. of tillers reduced*
Disomics	2.76	—0.20	8.8	— 7.7
Monosomics				
I	2.06	—0.68	18.9	30.5
II	2.23	—4.18	15.2	20.9
III	2.04	0.40	30.2	25.6
IV	1.97	2.20	17.5	— 0.2
VI	2.24	—1.70	19.6	27.6
VII	1.85	0.38	18.7	19.3
VIII	1.79	—0.08	17.8	44.6
IX	1.30	0.58	14.9	38.1
X	1.77	0.38	27.9	22.8
XI	1.71	0.80	14.9	13.2
XIII	1.70	3.50	21.0	30.8
XV	1.89	0.14	30.2	47.5
XVI	1.94	—0.42	27.3	42.8
XVII	2.18	—0.44	23.8	26.0
XIX	2.07	—0.42	20.4	47.7
XXI	1.90	—2.58	22.2	30.8
5% L.S.D.	0.10	3.43	10.5	30.8

* Values given in percentages.

TABLE 2

Correlation coefficients between 100-kernel weights and the radiosensitivities expressed on the germination percentage, seedling height, and number of tillers of the disomic and selfed progenies of 16 monosomics

	X_2	X_3	X_4
100-kernel weight = X_1	—0.399	—0.222	—0.465
Germination percentage decreased = X_2	....	0.114	—0.119
Seedling height reduced = X_3	....	....	0.522*
Number of tillers reduced = X_4	....	....	....

* Significant at 5% level.

ever, a significant correlation was obtained between the sensitivities observed in seedling heights at the first leaf stage and the number of tillers at the tillering stage.

Estimations of the X-ray effects on each of the "pure" monosomic populations (monosomic selfed progenies corrected for the number of monosomics expected) for the three characters studied, are shown in Figure 1. The relative radiosensitivities of the monosomic populations were not much different from those of the selfed progenies (see Table 1). Germination percentage of monosomic XIII was still reduced more than the others, while that of monosomic II was increased by irradiation. Monosomics X, XV, III, and XVI were more sensitive and the disomic was more resistant for effects on seedling height. Neither disomic nor monosomic IV showed serious effects of irradiation on tillering in comparison with a remarkable reduction of tillers in the other monosomics.

DISCUSSION

The completion of the four aneuploid series of common wheat, i.e., nulli-, mono-, tri-, and tetrasomics by SEARS (1954), opened several approaches to the study of the function of individual wheat chromosomes. Nulli- and monosomic analyses and the substitution method are the most widely used at present in wheat genetic studies. The nullisomic analysis (SEARS 1953) is based on the fact that different responses of the nullisomic plants from those of the disomic plants are attributed to the lack of the specific chromosome pair. Complete lack of a group of genes, however, probably interferes with a number of biochemical reactions necessary for normal plant development. Therefore, in the nullisomic condition the functional role of individual chromosomes at different developmental steps may not be properly evaluated because the upset of earlier developmental stages probably has serious effects on later stages of development. However, by irradiating a monosomic one may be able to determine whether a particular effect of the nullisomic is due to one or more genes. Chromosome II is known to promote awn growth in wheat but no chromosome II is now known which does not promote awns. Mutants obtained by irradiating chromosome II might be completely lacking in awn promoting capacity which would suggest a single gene, or if mutants occurred which lost only part of their awn promoting ability, more than one gene would be suggested.

Monosomic analyses, originally proposed by CLAUSEN and CAMERON (1944) in tobacco, have been applied in analyses of chromosomes responsible for various characteristics of wheat by many workers (UNRAU 1950; LARSON 1952; HEYNE and LIVERS 1953; CAMPBELL and MCGINNIS 1958; and others). The analysis, based on unusual segregation of characteristics in the filial generations of the specific monosomics, can be successfully applied only for characteristics that are different between two varieties involved in a monosomic cross. This approach has limited value in a study of the role of individual chromosomes on the more fundamental physiological plant functions common to all types of wheat.

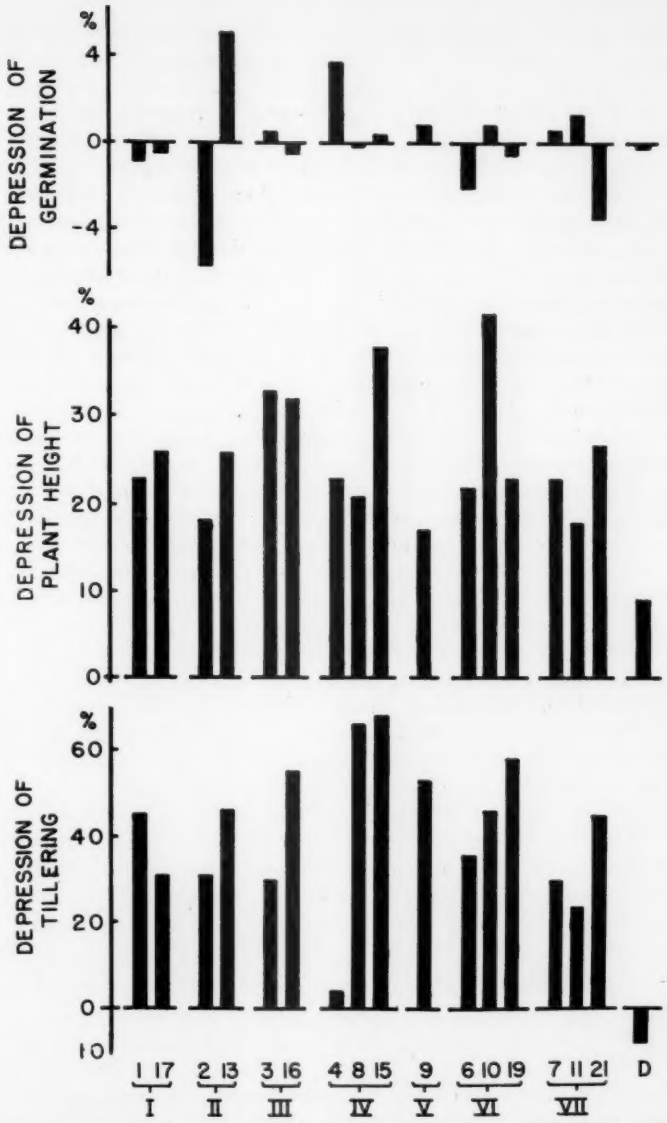

FIGURE 1.—Differential effect of X-rays on 16 "pure" monosomics and the disomic on germination (top), plant height in the seedling stage (center), and tillering (bottom) expressed as percentage decreases (+) or increases (−) for the characters studied. Arabic numerals are the corresponding monosomics; Roman numerals, the homoeologous groups; and D, the disomic.

The substitution method, suggested by SEARS (1953) and further advanced by UNRAU *et al.* (1956), consists of introducing a pair of chromosomes of a donor variety into a recipient variety by replacing the corresponding chromosome pair of the recipient through successive backcrosses of the donor variety with mono- or nullisomics of the recipient. A complete series of substitution lines gives information concerning which chromosomes are responsible for only certain characteristics that are different from the donor and recipient varieties.

In comparison with the approaches described above, a radiological study of monosomics with a moderate dosage of irradiation has the unique feature of being able to study the functional role of the individual chromosomes at various developmental stages. An embryo in a dormant seed consists of a number of cells. Moderate irradiation of the seed deletes or changes genes and/or their products in various cells. When a plant reaches a specific stage, a certain function may not be carried out in a group of cells because of injury to the gene or its products which are responsible for that development. This is expressed by an apparent radiological damage of the plant at that stage. At later stages of development of the same plant, other important functions may be blocked in different groups of cells expressing irradiation damage at those stages. However, there are generally a number of cells that function normally and allow the plant to recover from the specific irradiation damage. As the genes that are carried on a monosomic chromosome are in single dose, developmental steps for which these genes are responsible must be more seriously inhibited in the critical monosomic than in disomics. Such changes could occur without influences on later development. As a matter of fact, correlation values indicated that the differential radiosensitivity for the percentage germination was not significantly correlated with the differential sensitivities found in either of two later stages of development, i.e., with the seedling height and the number of tillers. However, the correlation between the last two was significant. The significant correlation can largely be attributed to the disomic, which showed the most resistance at both stages. The correlation coefficient calculated by excluding data on the disomic is 0.320 with d.f. $=14$, which is considerably short of significance at the five percent level. These considerations lead to the conclusion that radiological damage expressed on a developmental stage is independent from damage appearing at other stages of development. Thus, the radiological study of monosomics with a moderate irradiation is a useful approach to the study of functions of individual chromosomes in each developmental stage without a serious change of the whole process of development.

Comparisons of the functional roles of the homoeologous chromosomes in hexaploid wheat for each developmental stage should provide information on functional differentiation of these chromosomes that probably occurred during the course of evolution. Although the complete series of the monosomics was not involved in the experiment, the results provide some information on this problem. Monosomics II and XIII belong to homoeologous group II, and both showed extreme response to irradiation in percentage germination. Germina-

tion of monosomic II was stimulated and that of monosomic XIII inhibited. This suggested that both chromosomes are concerned with the process of germination but in opposite ways, e.g., chromosome II functions for dormancy of seeds and XIII for breaking of dormancy.

Two of the monosomics which showed extreme sensitivity at the development of the first leaf belonged to homoeologous group III, indicating that they have a similar function for leaf development of seedlings. This agrees with results reported by SEARS (1954) using nullisomic analysis. The monosomics belonging to homoeologous group IV showed response to X-rays on tillering development. Monosomic IV was most resistant but VIII and XV were sensitive, though their sensitivity was not significantly different from the average, indicating some functional divergency among these homoeologous chromosomes. However, SEARS (1954) observed an increased number of tillers in nullisomic VIII but not in IV. The disagreement of the results on tillering must be studied further. Although these results do not agree exactly with SEARS (1954), they agree in that homoeologous group IV functions by affecting tillering.

Concerning the principles involved, moderate irradiation of monosomics in hexaploid wheat facilitates study of chromosome functions in a rather normal physiology of the plant; it may be applied to other polyploid species and may be useful if trisomics of diploid species are irradiated.

SUMMARY

Comparative studies of radiosensitivity of disomic and selfed progenies of 16 monosomics of hexaploid wheat were made at three stages of development after irradiating dormant seeds with 14 Kr units of X-rays. The differential radiosensitivity statistically defined in the previous paper (TSUNEWAKI and HEYNE 1959b) was significant for percentage germination, highly significant for seedling heights at the first leaf stage, and significant for number of tillers produced at the tillering stage. Correlation studies and covariance analysis indicated that the difference of seed size found was not responsible for the differential sensitivity observed.

Germination rates of monosomics II and XIII, both of which belong to homoeologous group II, were most increased and reduced, respectively, indicating a functional divergence of the corresponding homoeologous chromosomes on germination. X-ray damage on the seedling height was most pronounced in monosomics X, XV, III, and XVI. Monosomics III and XVI belong to homoeologous group III, indicating a similar function of the corresponding chromosomes on the leaf development of seedlings. Monosomics IV, VIII and XV, all of which belong to homoeologous group IV, were concerned with tillering. Monosomic IV was significantly resistant to irradiation effects while the other two were sensitive.

Radiological studies of monosomics exposed to a moderate dosage of irradiation appears to be a useful approach to the study of specific functions of individual chromosomes at various stages of development from the standpoint of develop-

mental genetics on one hand and the investigation of functional homology and divergence among the homoeologous chromosomes from an evolutionary viewpoint on the other hand.

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ADDITIONAL ALLELES AFFECTING RED BLOOD CELL ANTIGENS IN THE CHICKEN[1]

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FOLLOWING the report by BORDET (1898) that the serum of one species of animal would often agglutinate the cells of other species, other workers observed individual differences in the cellular antigens of man (LANDSTEINER 1900), goats (EHRLICH 1900) and cattle (TODD and WHITE 1910). LANDSTEINER and MILLER (1924) detected individual differences in the cellular antigens of chickens by absorbing antichicken serum produced in rabbits with the blood cells of individuals. TODD (1930) produced isoagglutinins by immunizing one chicken with the blood of another, and as a result of further experiments proposed (1931) that there existed individuality of the blood cells of a species. However, both KOZELKA (1933) and WIENER (1934) postulated that a restricted number of antigenic substances could explain the results of TODD.

Two multiple allelic series of genes affecting cellular antigens of the chicken were described by BRILES, McGIBBON and IRWIN (1950). Evidence was presented for the existence of nine alleles at the "*A*" locus, and for five alleles at the "*B*" locus. A few tests for linkage of genes at either locus with the gene for dominant white (*I*) disclosed no significant association. However, BRILES, C., BRILES and QUISENBERRY (1950) noted a crossover frequency of 39.8 percent between the gene for crest (*Cr*) and one affecting an antigenic substance called X_3, which seemingly belonged in the A system. Since the genes *I* and *Cr* are linked with 12.5 crossover units (WARREN and HUTT 1936), linkage of the genes in the A system and *I* should be demonstrable. Two linkage groups of genes for cellular antigens in the chicken have been described by SCHEINBERG (1956), one of which may belong to the A system of BRILES, W., BRILES and QUISENBERRY (1950). Two other loci, *D* and *E*, with genes affecting cellular antigens in chickens have been reported by BRILES (1951 and 1956). The present evidence suggests that the *A* and *E* loci are linked by about one crossover unit or else they are one system controlled by a complex single blood group locus (BRILES 1958).

This paper describes the serological and genetic relationship of chicken red blood cell antigens produced by genes at the *A*, *B*, *D* and *E* loci. Additional alleles have been demonstrated at the *A* and *E* loci which provide more data on the relationship of the two systems.

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MATERIALS AND METHODS

The general procedures were similar to those described by BRILES, MCGIBBON and IRWIN (1950). The immune sera were produced by the injection intravenously of one cc of citrated whole blood or a 50 percent cell suspension at three day intervals until a satisfactory titer was observed. Maximum yields of serum from the clotted blood were obtained by filling dry test tubes (16 mm × 120 mm) approximately half full of blood, and allowing these to stand in a slanting position. After several hours at room temperature and following centrifugation and inactivation at 56°C, the antisera were stored at $-20°C \pm 5°C$. The respective reagents used in this study were prepared by differential absorption of the isoimmune sera. (The details of the different absorptions will be furnished upon request.)

The agglutinations were performed in the manner described by BRILES, MCGIBBON and IRWIN (1950). The readings of the agglutinations were made after the cell-reagent mixture had incubated for approximately two hours at room temperature, and again after storage in a refrigerator overnight. All questionable reactions and the negative controls were checked microscopically.

Terminology

The blood group systems dealt with in this study are represented by the letters A, B, D and E. The capital letters designate the system to which the antigen or specificity belongs. An agglutinogen or red cell antigen is indicated by a letter followed by a subscript designation (e.g., A_1) in contrast to the superscript symbol assigned to the gene (e.g., A^1). The reagents employed in testing the red cells of the progeny from the various families are designated by a number or numbers prefixed by a capital (e.g., A1).

When an antibody fluid or reagent was first prepared, it was assigned a temporary number until the locus of the gene responsible for the reactive agglutinogen could be determined.

Preparation of reagents

The various reagents were prepared by absorbing an antiserum individually with the reactive red cells of birds chosen either at random or because of their content of antigenic substances. Each antiserum so absorbed (reagent or test fluid) was then tested with the cells of selected birds, the objective being that each reagent should be reactive with a single antigenic specificity. To illustrate the general procedure for the preparation of reagents the analysis of one antiserum will be given.

The serological analysis of antiserum J1057 (J1053), which was produced by injecting the whole blood of bird J1053 (the donor) into J1057 (the recipient), is shown in Table 1. The birds were Single Comb White Leghorns (S.C.W.L.) of Wisconsin's inbred line 3. The cells of the donor J1053 reacted with the reagent for D1 (Texas), obtained by courtesy of W. E. BRILES, while those of the recipient J1057 did not. The antibody titer of this antiserum, J1057 (J1053), was 1:64. When this antiserum was absorbed by the weakly reactive cells of J1063

TABLE 1

Differential absorption analysis of antiserum J1057 (J1053), showing the presence of four specificities of the antibodies

Row	Absorbed by cells of:	Postulated: Antigens in absorbing cells				Postulated: Agglutinins remaining in serum				Agglutinations with the cells of: J1053	J1057	J1063	J1065	J1066	J1069	J1272	J1316	B3440	E1311	E712	E777	S21	A2282	A2283	E753	C2707	A952	J5	J6
										Proposed antigens of test cells of: D1 W_1 A_7 A_8	None	W_1	D_1	D_1	D_1	D_1	D_1	W_1	A_7 A_3	D_1 A_3	D_1 A_7 A_8	D_1 A_7	D_1 A_7	D_1 A_7 A_8	D_1 A_8	D_1 A_8	D_1 A_7	D_1 A_7	A_7	A_7
1.	unabsorbed	..	..	..	..	D1	W1	A7	A8	+	0	†	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	J1063	..	W_1	..	..	D1	..	A7	A8	+	0	0	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+
3.	J1063	..	W_1	..	..	..	..	A7	A8	+	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+
	J1066	D_1	..	..	..																								
4.	J1063	..	W_1	..	..	..	..	A7	..	+	0	0	0	0	0	0	0	0	+	0	+	+	+	+	0	0	+	+	+
	E753	D_1	..	..	A_8																								
5.	J1063	..	W_1	..	..	..	..	..	A8	+	0	0	0	0	0	0	0	0	+	+	+	0	0	+	+	+	0	0	0
	A952	D_1	..	A_7	..																								
6.	J1063	..	W_1	..	..	D1	..	..	..	+	0	0	+	+	+	+	+	0	0	+	+	+	+	+	+	+	0	0
	E1311	..	..	A_7	A_8																								
7.	J1063	..	W_1	..	..	None																							
	E753	D_1	..	..	A_8	..	..	..	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A952	D_1	..	A_7	..																								
8.	D1 Reagent (Texas)	..	..	..	..	D1	..	..	..	+	0	0	+	+	+	+	+	0	0	+	+	+	+	+	+	+	0	0

Symbols: + = strong agglutination; † = weak agglutination; 0 = no agglutination. Birds J1053 through B3440 are S.C.W.L., E1311 is an F_2 (N. H. × S. C. W. L.) and A2282 and A2283 are B.P.R.; all other birds with the prefixes A,C,E,J, and S are N. H.

of Wisconsin inbred line 3, agglutinins were removed only for the absorbing cells and for the cells of B3440. The antigenic component of the cells of J1063 is called W1. The reactions of this reagent with the cells of the Leghorns paralleled those of the D1 reagent (Texas) as is shown in rows 2 and 8 of Table 1. However, when this reagent for D1 (Wisconsin) was tested with cells of various birds of the New Hampshire (N.H.) breed which were not reactive with the reagent for D1 of Texas, definite agglutinations occurred (birds J5 and J6). In agglutinations following absorption by cells of J1063 and those containing the antigen D_1, as J1066 (row 3 of Table 1), antibodies still remained which reacted with the cells of other breeds and some S.C.W.L., including those of the donor, J1053. This implies that at least one additional antibody other than the W1 (unidentified, in reference to the A, B, D and E systems) and D1 is present in the serum.

Absorption with the pooled cells of J1063 (containing W1) and E753 (containing D1 and at least one other specificity, as indicated in row 4 of Table 1) provided a reagent which was reactive with the cells of the donor J1053, and several birds of breeds other than S.C.W.L. Tests to be described later indicated that the gene producing the antigen recognized by this reagent segregated independently of D^1, and identified it as an allele belonging to the *A* locus. By testing the cells of backcross progeny from parents of known genotype this substance was found to be A^7. The absorption of this antiserum with the cells of J1063 and A952 (as shown in row 5 of Table 1) produced a reagent with at least one specificity other than W_1, D_1, and A_7, which was reactive with the cells of various birds, including the donor J1053. This absorption removed all antibodies except those recognizing another specificity which was produced by a gene identified (by testing cells of progeny from the appropriate testcrosses) as an allele also belonging to the *A* locus, A^8. This antigenic specificity behaved as a unit substance, as will be shown later. The respective reagents for the A_7 and A_8 antigens were reactive with the cells of E1311 (an F_2 from a N.H. × S.C.W.L. cross), hence the cells of J1063 (W_1) and E1311 (A_7 and A_8) were pooled and mixed with this antiserum; the antibodies remaining paralleled those of the D1 reagent (Texas) in agglutination of the cells of all birds tested (rows 6 and 8 of Table 1). Finally, the absorption of the antiserum by the pooled cells of J1063 (W_1), E753 (A_8 and D_1) and A952 (A_7 and D_1) removed all antibodies from the antiserum (row 7 of Table 1). The cells tested with the absorbed fluids of the antiserum J1057 (J1053) constituted six classes: those possessing antigenic substances D_1; A_7, D_1; A_8, D_1; A_7, A_8; A_7, A_8 and D_1; and those not reacting.

The additional reagents that were used in these experiments were prepared and tested for their specificities in a manner similar to that discussed for the antiserum presented in Table 1. The origin of at least one source of each of the additional reagents used in this study is shown in Table 2.

To determine the system specificity of the various reagents and to identify the particular specificity within blood group systems (A, B, D or E), the cells of birds of known blood type were tested with each of the newly prepared reagents.

The antigenic substances A_7, A_9, B_2, B_8, D_1, E_1, and E_2 had been identified in

TABLE 2

Source of additional reagents

Reagent	Recipient		Donor	
	Bird number	Breed	Bird number	Breed
A6	C2325	N.H.	C98	N.H.
A68	C2325	N.H.	C98	N.H.
A9	C4537	W.L.	J1079	W.L.
B2	C4913	W.L.	C4912	W.L.
B28	A950	W.L.	M770	W.L.
E1	A4261	W.L.	M776	W.L.
E12	6969	W.L.	A3352	B.P.R. × N.H.
E24	I1086	W.L.	C4920	W.L.

three inbred lines of S.C W.L. prior to this study by DR. W. E. BRILES of Texas A. and M. College (BRILES, W., BRILES and QUISENBERRY 1950; BRILES, MCGIBBON and IRWIN 1950; BRILES 1951; BRILES, FANGUY, KRUEGER and QUISENBERRY 1956). The gene products A_6 and A_8 have not previously been reported, as well as certain combinations of A_6 and A_8 with A- and A_9.

The A8 and A68 reagents were prepared from two different antisera. The A6 reagent was in turn prepared from the A68 reagent by absorbing it with cells possessing the A_8 antigen. An A8 reagent could not be made from the A68 reagent in this particular study because all cells containing A_6 also carried one or more specificities of the A system.

GENETIC RESULTS

As mentioned previously, each reagent was designated by a different number preceded by the letter W on the assumption that the different reagents were reacting with antigenic substances inherited independently. After the data on genetic segregation were obtained (Tables 3, 4 and 5), evidence was considered for (1) the unitary or multiple specificities of the reagents prepared and (2) the mode of inheritance of the various antigenic substances or complexes detected by the various reagents.

In Table 3 are given the agglutination reactions of the cells of a sire (A8688) and a dam (M763), and the four classes of their progeny with the reagents A6, A7, A8, A68 and A9. Of the 31 progeny from this mating, there were nine whose cells were reactive with each of the five reagents, eight whose cells were reactive with four of the five reagents but not with that for A7, seven whose cells were reactive only with the reagents A7 and A9, and seven whose cells were reactive only with the reagent for A7. The phenotype of the birds of the first class was A_6, A_7, A_8 and A_9, and their probable genotype was A^{689}/A^7. The birds of the second group were A_6, A_8 and A_9, and their probable genotype was A^{689}/A^9; those of the third group carried the antigenic specificities A_7 and A_9, their probable genotypes being A^7/A^9; those of the fourth group carried only A_7, and their genotype presumably was A^7/A^7.

The cells of the 17 progeny of the first and second classes were agglutinated by the respective reagents for A6, A8 and A9, and those of the seven birds of the third class were reactive with the A9 reagent but not with A6, A8 or A68. That is. the antigenic specificities A_6, A_8 and A_9 behaved as a unit if A_6 and A_8 were

TABLE 3

The agglutination reactions of the parents (A8688 × M763) and progeny in tests with reagents for antigenic specificities of the A system

Cells of	Number of progeny in each group	Presence or absence of agglutination of the cells with the respective reagents					Phenotype	Probable genotype
		A6	A7	A8	A68	A9		
A8688	.	+	+	+	+	+	A_6,A_7,A_8,A_9	A^{689}/A^7
M763	.	0	+	0	0	+	A_7,A_9	A^7/A^9
Progeny	9	+	+	+	+	+	A_6,A_7,A_8,A_9	A^{689}/A^7
	8	+	0	+	+	+	A_6,A_8,A_9	A^{689}/A^9
	7	0	+	0	0	+	A_7,A_9	A^7/A^9
	7	0	+	0	0	0	A_7	A^7/A^7

Symbols: + and 0 indicate presence or absence of agglutination of the cells with the respective reagents. The reactions of the cells of each of the 31 progeny will be furnished if requested.

TABLE 4

The distribution among the progeny, from various matings of the antigenic specificities of the A, B, and D antigenic systems of the cells of chickens

A. Antigenic specificities of the A system

Mating number	Number of offspring possessing the antigenic specificities								Probably genotypes of the parents
	a	A_6	A_8	A_{68}	A_{78}	A_9	A_{79}	A_{689}	
1	14	13	..	..	..	..	..	..	♂ $A^{6(89)*}/A^{(7)}$ × ♀ a/a
2	11	..	9	..	..	..	..	..	♂ $A^{(6)8(9)}/A^{(7)}$ × ♀ a/a
3	29	..	..	..	..	..	..	25	♂ $A^{689}/A^{(7)}$ × ♀ a/a
4	..	..	5	..	..	6	..	..	♀ A^8/A^9 × ♂ $A^{(7)}/a$
5	5	..	..	10	..	..	..	..	♀ $A^{68}/A^{(9)}$ × ♂ a/a
6	27	..	..	..	27	..	..	..	♀ $A^{78}/A^{(9)}$ × ♂ a/a
7	..	..	..	..	18	..	18	..	♀ A^{79}/A^{78} × ♂ A^7/a

B. Antigenic specificities of the B and D systems

Mating number	Number of offspring possessing the antigenic specificities						Probable genotypes of the parents
	b	B_2	B_8	B_{28}	d	D_1	
8	33	31	..	..	..	..	♂ B^2/b × ♀ b/b
9	22	..	16	..	..	..	♂ B^8/b × ♀ b/b
10	16	..	..	19	..	..	♂ B^{28}/b × ♀ b/b
11	..	13	12	..	..	..	♂ B^2/B^8 × ♀ b/b
12	..	..	..	..	26	28	♂ D^1/d × ♀ d/d

* Digits enclosed in parenthesis indicate the reagent detecting that particular antigenic specificity was not used in testing the cells of the progeny.

TABLE 5

The distribution of the progeny resulting from different matings when tested with the respective E and A reagents

Mating number	Number of offspring possessing antigens									
	E System					A System				
	e	E_1	E_2	E_4	$E_{2,4}$	a	A_6	A_7	A_8	A_9
1	9	10	..	..	..	..	..	..	..	..
2	..	..	4	16	5	..	16	9	16	16
2′	16	..	9	..	..	..	16	9	16	16
3	2	3	..	..	..	3	..	2	..	..
4	5	6	..	..	..	..	..	..	5	6
5	14	6	..	..	..	6	..	14	..	..

present, whereas A_9 occurred either in the presence or in the absence of the other two components. Hence presumably the genotype of A8688 was A^{689}/A^{7}, and that of M763 was A^{7}/A^{9}.

Evidence is presented in Table 4 which indicates that the antigenic substances found to be reactive with the various reagents are frequently inherited as intricate combinations. The progeny of sire A8688 from matings 1 (dam M762) and 2 (dam M770), when only using one reagent for testing the cells of the offspring from each dam, antigens A_6 and A_8 respectively, show a 1 to 1 segregation of a single gene producing an antigen and its allele producing no detectable antigen. The progeny from matings 1 and 2 consisted of three classes: those reacting with the A6 reagent (13 birds), with the A8 reagent (nine birds), and those not reacting with either reagent (14 and 11 from matings 1 and 2 respectively), Table 4. The progeny from mating 3 were divided approximately equally into (a) those (29) whose cells did not react with any of the reagents of the A system, and (b) those (25) whose cells reacted with the respective reagents for A6, A8 and A9. In these progeny the A_6, A_8 and A_9 specificities behaved as a unit as was also indicated in the data of Table 3.

In mating 4 of Table 4, the dam (B7881), whose cells reacted with both the A8 and A9 reagents, was mated to a sire (A921) whose cells contained A_7 but were nonreactive with the A8 and A9 reagents (genotype A^{7}/a). The progeny from this mating were tested with only two reagents, A8 and A9; on the basis of these tests, two classes of offspring were detected—five chicks whose cells reacted with the A8 reagent and six whose cells were reactive with the A9 reagent. Thus, the A8 and A9 specificities behaved as antithetical characters in the progeny, showing that the genotype of the dam was A^{8}/A^{9}.

In mating 5, the cells of the dam (B7913) also reacted with two reagents, A6 and A8. The progeny were of two classes, five whose cells were not agglutinated by either of the A reagents (A6 and A8) and ten whose cells reacted to both. The proposed genotype of this dam is A^{68}/A^{9}, but the progeny were not tested with the A9 reagent.

In mating 6, the cells of dam K1899 were reactive with the A7, A8 and A9

reagents while those of the sire were not. The cells of half the 54 progeny were reactive with reagents A7 and A8, those of the other half with neither, indicating that the specificities A_7 and A_8 were a unit in inheritance. Thus, the genotype of the dam was postulated to be A^{78}/A^{9}. The reagent for A9 was not tested on the cells of the progeny.

Mating 7 illustrates another antigenic complex of the A system. The cells of dam K1893 reacted with the A7, A8 and A9 reagents. Here again, two classes of progeny resulted—18 whose cells reacted with the A7 and A8 reagents and 18 with cells reactive with the A7 and A9 reagents. The sire (A921) was known to be of the genotype A^{7}/a through progeny tests on other dams. The occurrence of the A_7 substance among all 54 progeny indicates that both alleles transmitted by the dam were producing the A_7 specificity. Therefore, the genotype of the dam (K1893) is proposed to be A^{79}/A^{78}.

The data of part B of Table 4 show the segregation of antigens (B_2, B_8 and B_{28}) of the B system, and D_1 of the D system, in matings in which only one parent possessed the demonstrable antigen or antigens. (Attempts to produce antibodies which would be reactive with antigens other than D_1 of the D system were not successful.) The progeny from each mating were divided approximately equally according to the presence or absence of the antigenic specificities: in mating 8, there were 31 with B_2 to 33 lacking it; in mating 9, there were 16 with B_8 to 22 without; in mating 10, there were 19 with the complex B_{28} to 16 without; in mating 11, there were 13 with B_2 to 12 with B_8; and in mating 12, there were 28 with D_1 to 26 without. These results indicate the antithetical nature of the B_2 and B_8 antigens, since in mating 11 they separated as expected if the causative genes were alleles. The progeny of mating 10 show that another gene, presumably of the same allelic series, may affect both B_2 and B_8 specificities. Parallel findings have been made in the chicken (BRILES, MCGIBBON and IRWIN 1950) and in cattle (STORMONT, OWEN and IRWIN 1948), and have led to the concept that a gene may affect multiple antigenic specificities of a cellular antigen.

It is apparent that the E system in chickens is produced by genes which are (a) closely linked to the alleles at the *A* locus or (b) are alleles at the *A* locus. BRILES (1958) has reported that the *A* and *E* loci are linked by about one crossover unit. The data in Table 5 confirm the close relationship of the two systems, but do not show complete evidence as to linkage or allelism of the causative genes.

The progeny from mating 1 of Table 5 were of approximately equal numbers according to the presence or absence of E_1; there were ten birds whose cells were reactive with the E1 reagent, nine whose cells were not reactive. The probable genotypes of the parents are $E^{2}/e \times E^{1}/e$. Cells of the progeny were tested with the E1 reagent, but not with that for E2. Evidence for the identification of a new agglutinin called E4 is presented in mating 2 of Table 5. These data show the distribution of progeny from two dams, M732 and M737 (E^{4}/e) and the sire A8688 (E^{2}/E^{4}). Based on the proposed genotypes of the parents, the progeny from this mating would consist of four genotypic classes: E^{2}/E^{4}, E^{2}/e, E^{4}/E^{4} and E^{4}/e. The cells of these 25 progeny were tested with the E12 and E24 reagents,

resulting in nine birds whose cells reacted with both E12 and E24 (genotypes E^2/E^4 and E^2/e), and 16 whose cells reacted with E24 alone (genotypes homozygous and heterozygous for E^4). The nine positive birds were divided arbitrarily into four possessing the E_2 antigen and five with $E_{2,4}$ on the basis of chance expectation. Thus these 25 progeny may be classified as four of genotype E^2/e possessing the E_2 antigen, five of genotype E^2/E^4 possessing the E_2 and E_4 antigens, and 16 of genotypes E^4/E^4 and E^4/e (also divided in approximately equal numbers of homozygotes and heterozygotes) possessing the E_4 antigen only. The nine individuals whose cells were reactive with the E12 reagent were also agglutinated by the A7 reagent and those of the 16 progeny not reactive with the E12 reagent did react with the reagents for A6, A8 and A9 (see mating 2′). The probable genotypes of these parents with respect to the E and A systems are $\delta\ E^4/E^2\ A^{689}/A^7 \times \varphi\ E^4/e\ a/a$.

Besides the data from mating 2′, the offspring of matings 3, 4 and 5 of Table 5 provide additional evidence of a relationship of the A and E systems. Thus, two of the five offspring of mating 3 which did not carry the E_1 antigen possessed A_7. Of the 11 offspring from mating 4, there were six individuals with E_1 and A_9, and the five birds without E_1 carried A_8. The probable genotypes of the parents of mating 4 are $\delta\ E^{(24)}/e\ A^{(7)}/a \times \varphi\ E^1/e\ A^{(7)8}/A^9$. The cells of the offspring were not tested with the E24 and A7 reagents, as indicated by parenthesis around the digit in the genotype of the parents. In number 5 mating, six of the 20 progeny carried E_1, the other 14 sibs possessed A_7 but not E_1. The probable genotypes of these parents are $\delta\ e/e\ a/a \times \varphi\ E^1/e\ A^7/a$. From each of the five above matings (Table 5) there were four genotypic classes of offspring expected if independent loci were involved, but only two classes were observed. These data, representing a total of 61 segregants, are explicable on the basis of a single locus; however, with linkage of approximately one percent as reported by BRILES (1958), these data are fully within chance expectation and are thus compatible with the concept of close linkage between the *A* and *E* loci.

DISCUSSION

Testcross progeny were utilized in establishing the genetic relationship of the effects of the blood group genes represented by the various reagents prepared for this investigation. The findings in this report are additional evidence for the proposal that the antigenic products of many, perhaps all, of the genes affecting cellular antigens have multiple antigenic specificities. (For references to previous articles advancing this proposal, see BRILES, MCGIBBON and IRWIN 1950; MCGIBBON 1944 and STORMONT 1955). For example, the 198 progeny of male A8688 were divided approximately equally into those birds with and those without the antigenic character A_{689}. Further, the antithetical relationship in other individuals of one or more of this combination of antigenic specificities (A_8 and A_9, A_{78} and A_{79}, and A_{68} or A_{78} presumably with A_9 as in Table 3) are readily interpreted on the basis of their production by genes forming a series of multiple alleles.

Cross-reactivity of antigens is based upon the assumption that separate specificities are produced by different alleles at any one locus and because of their similarities, cross-reaction occurs. This may explain the multiple specificities of supposedly one allele (A^{689}). However, the A system produced no apparent cross-reactivity as did the B system. Also, one cannot exclude entirely the possibility that pseudoalleles may be involved, because intensive tests of multiple allelic systems have generally, and may always, reveal the presence of closely linked loci. Such findings have been made by LEWIS (1945) and GREEN and GREEN (1949) in Drosophila, and by STEPHENS (1948) and YU and CHANG (1948) in cotton, to cite only a few papers bearing on this subject. However, until crossing over has been demonstrated, it would seem reasonable to accept the hypothesis that multiple alleles affect the antigenic characters in the different antigenic systems of the chicken.

SUMMARY

Genetic studies were carried out on the red blood cell antigens of the chicken. The antigens were identified by reagents or test fluids which contained antibodies prepared from isoimmune sera, usually by differential absorption with erythrocytes of selected individuals. The antigenic substances, their causative genes, and the reagents (containing agglutinins) that detect them are designated by specified terminology.

The reagents were used in testing the cells of progeny from testcross matings in order to determine the genetic relationships of the antigens and their causative genes. Where more than two alleles were involved in a mating, the serological tests were not always adequate to differentiate all genotypes. The reagents identified antigens produced by genes at four autosomal loci— *A*, *B*, *D* and *E*. There is suggestive evidence that the *A* and *E* loci are associated in close linkage.

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THE RELATIONSHIP BETWEEN RADIATION DOSE AND DOMINANT VISIBLE MUTATION RATE IN DROSOPHILA MELANOGASTER[1]

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IT has recently been shown (IVES 1958, 1959) that over a broad dosage range, extending well above previously studied dosage levels of Cobalt-60 γ radiation, the relationship between rate of sex-linked lethal mutations and radiation dose is apparently linear. Concurrently tests were made of the frequency of easily seen visible mutations, chiefly autosomal dominants, at ten dosage levels in the .5 kr to 10 kr range. The purpose of this report is to present the results of those tests and to relate them in particular to the findings in the sex-linked lethal tests.

MATERIALS AND METHODS

The stocks used in this test were the same highly inbred Oregon-R wild type which was used in the X-lethal tests, and the marker stock known as *hes* carrying seven recessive third chromosome visible mutations. The Oregon-R stock had been inbred for 150 generations when the tests with *hes* began, but was mass mated during the period of the tests. A new derivative from the inbred Oregon-R line was substituted every 20 to 25 generations in order to minimize the effects of cumulative variability in the stock. It proved impractical to inbreed the *hes* stock, which was carried in small mass matings of about ten pairs of parents. Occasional clusters of mutations appeared during the experiment. They were usually traceable to the *hes* stock. That indicates the desirability of using inbred stocks in this kind of study whenever it is possible to do so.

The *hes* mutants are hairy, thread, scarlet, peach, curled, stripe, sooty. The basic symbols for those loci are *h*, *th*, *st*, *p*, *cu*, *sr*, *e*. They are described in BRIDGES and BREHME (1944).

In the general procedure of these tests, wild type males were raised at 25°C and were collected from the earliest flies emerging in a culture, within 12 hours after eclosion. Aged then on well yeasted medium for two days separated from females, they were irradiated with Cobalt-60 γ rays from a source which contained 430 curie at the beginning of this work. Intensities of radiation ranged between 100r and 250r per minute. Adjustments were made weekly for isotope degradation. Immediately after the treatment the males were mated for three days to *hes* females. For doses under 6 kr (the indicated dose is that which was delivered to the surface of the fly) the mating consisted of one male to two or

[1] This work was done under Contract AT(30–1)–930 with the United States Atomic Energy Commission.

three females. For doses of 6 kr and higher the mating was two or three males with four or five females.

The F_1 of these matings were raised in 95 × 25 mm shell vials containing about 10cc of a standard medium made from cornmeal, molasses, brewers yeast and agar. The medium contained methyl parasept as a mold preventative and was dusted very lightly with a few particles of powdered live yeast. Overcrowding of larvae in controls and tests of low doses was minimized by the use of only two female parents which were shaken to fresh cultures as often as was needed, generally daily. To increase the yield in tests of high doses the females were shaken to a second culture for an additional laying period at the time when the male parents were removed.

Control and low-dosage tests were carried out simultaneously and were randomized so that the F_1 could be classed without a bias toward either type of test. In tests of higher levels of radiation, three or more adjacent dosages were randomized similarly. The different dosage levels were tested alternately in time in order to minimize variations due to possible shifting classification standards. All classing was done by the author.

F_1 flies were examined under 9 × 2.3 binocular magnification and included all flies emerging through the 18th day after egg laying began. All the offspring from one male parent, or from a group of male parents, were tabulated together in order to detect clusters of mutant flies whose mutation was not related to the radiation treatment. All members of a cluster were examined routinely for the presence of any other visible mutation.

The F_1 were scored for any bilateral abnormal phenotype which was noticed while examining the flies for mutations of the *hes* group. Only wing venation was examined in addition to the parts affected by the *hes* genes. An abnormal phenotype had to be describable in specific verbal terms in order to be scored as mutant.

Most commonly found were variants of the small bristle phenotype called Minute, loci for which are distributed through all the major euchromatic areas. Minutes constituted 50 percent of the mutant phenotypes found in the radiation tests. Mutants of the *hes* group amounted to only 14 percent of the total. The remaining 36 percent were a wide variety of phenotypes which have been classed under one general heading, "other bilaterals." They will be referred to as OB.

About 20 percent of the OB were phenotypes closely resembling Delta (*Dl*), Hairless (*H*) and aristopedia (ss^a). Other well known phenotypes encountered repeatedly were sex-linked Notch (*N*) and second chromosome Plum (bw^V).

In addition to all of these mutations, another class, unilaterals or partially mutant flies, was recorded. It proved impossible to score such cases with a consistency approaching that achieved in scoring bilateral mutants. Therefore, no data will be presented for them at the present time.

Most of the *hes* mutants were tested for simple allelism (not pseudoallelism) by backcrossing to *hes*. Many of the more "attractive" OB, including the five specific types mentioned above, were tested for their dominant inheritance and

linkage group. When the mutant flies were fertile, which they were about half the time but less so when occurring in tests of doses above 5 kr, allelism was nearly always indicated for the *hes* mutants and dominant inheritance for the OB. Rarely was there positive evidence of a dominant mimic mutation in the tests of *hes*-like mutants. When the tested OB were females and when a mutation was linked to the third chromosome, it was generally observed that crossing over was reduced or absent in the region carrying the mutation.

It was not possible to make careful systematic tests of all mutant F_1 flies. It requires rigidly controlled environmental conditions to determine critically by such tests the proportions of sterile *vs.* fertile, allelic *vs.* nonallelic, and hereditary *vs.* nonhereditary mutants. An example of one uncontrolled variable in the present tests which would at least have weakened the value of such systematic tests in this work was the condition of the culture medium in which the F_1 larvae grew. It was possible to minimize crowding effects in controls and low doses, but at high doses the larval population was usually well below optimal size so that excessive drying and darkening of the medium occurred before the larvae pupated. While no evidence was obtained that this food condition produced phenocopies among the F_1, it may well have been a factor in the fertility and fecundity of the F_1, in particular the mutant F_1. It is not known what net effect such conditions may have had on the rate of recovered mutant flies, but at least there was no evidence of any qualitative difference in mutants observed after high and low doses. There was such a difference in the controls, which produced none of the *hes* mutants and a few of the types of OB observed in radiation series.

In view of the above considerations, it seems best to the author to calculate mutation rates in this study on the basis of the F_1 scoring.

TABLE 1

Rates of visible mutations after γ-radiation

Dosage in kr	No. flies	Number and percent of major classes*								Increase over control
		hes	%	*M*	%	OB	%	Total	%	
0	50,970	0	.00	61	.12	25	.05	86	.17	..
.5	34,241	8	.02	64	.19	39	.11	111	.33	.16
1	22,103	20	.09	68	.31	40	.18	128	.58	.41
2	15,420	19	.12	85	.55	45	.29	149	.97	.80
3	13,640	33	.24	117	.86	75	.55	225	1.65	1.48
4	10,338	35	.34	116	1.12	74	.72	225	2.18	2.01
5	4,438	18	.41	55	1.24	68	1.53	141	3.18	3.01
6	4,331	28	.65	103	2.38	64	1.48	195	4.50	4.33
7.5	3,519	26	.75	95	2.70	71	2.02	192	5.46	5.29
9	2,041	21	1.03	67	3.28	56	2.74	144	7.06	6.89
10	2,361	25	1.06	83	3.52	87	3.68	195	8.26	8.09

* Description of mutant classes:
hes = *h*, *th*, *st*, *p*, *cu*, *sr*, *e*.
M = Minutes.
OB = Other bilaterals.

Presentation of data

The frequencies and rates of F_1 mutant flies in these tests are given in Table 1. Included are the number of F_1 flies classed, the number and percent of each of the three major classes of mutations observed and the total number and percent of mutant flies. The last column shows the increase over the total control value in the total percent of mutant flies in each radiation dosage test. That represents the net increase due to the effects of radiation.

Two numerical criteria have been met at each radiation dosage test: at least 2000 flies classed and at least 100 mutants scored. The goal in the controls was a minimum of 50,000 flies from noncrowded cultures. About as many more control flies have been excluded because of overcrowded cultures in most of the control series in the early part of the work. Overcrowding is indicated when a large portion of the F_1 emerge between days 14 and 18. In optimal conditions the large majority of flies emerge in control and low dosage tests between days 10 and 14 after the beginning of development. When it became apparent that only under such optimal conditions were Minutes obtained at the indicated frequency (it decreases sharply under overcrowded conditions), all cultures indicating overcrowding were excluded from control and low dosage series. Overcrowding was not observed in tests of doses above 1 kr.

Had *hes* mutants appeared in the control tests in the same proportion as in the 1705 radiation series mutants, there would have been some ten of them among the 85 control mutants. That they do occur, however, without known radiation treatment is reasonably certain. The author has found alleles of most of these mutant loci in chromosomes extracted from natural populations, and has observed *de novo* mutations for *h* and *th* in other wild type stocks which had a long history of inbreeding. The control data in Table 1 indicate only that 50,000 chromosome tests is an insufficient sample to measure the mutation rate for the *hes* loci, even as a group.

As data accumulated in the control tests it became apparent that a few abnormal phenotypes which are not individually hereditary occur repeatedly in flies produced by the particular cross of Oregon-R to *hes*, probably as developmental anomalies. Flies of these specific types have been excluded from the OB column in all of the entries in Table 1. Most of the control OB, in contrast to those in radiation series, proved to be nonhereditary, and could have been less frequently occurring development anomalies than the types which were arbitrarily excluded from all series. Three of the hereditary control OB, involving different phenotypes, were found as clusters of mutant flies and were traced to the *hes* stock. Several similar cases appeared in the radiation series. In each instance the mutation was scored as only one mutant, in the culture of flies first showing it, and only *hes* cultures free of the mutant were used thereafter.

Tests of some of the control Minutes indicated that about half of those were cases of haplo-4, the loss of one fourth chromosome. Each of the other autosomes contributed some Minutes to the control group, including the *hes* marker chromo-

some itself. The haplo-4 phenotype was much less common among the radiation Minutes.

The data of Table 1 show that the rate of each of the three classes of mutations increased with dose. The rate of increase, given in the final column, is more than linear when the total mutation rate is considered. This can be seen by comparing the mutant percentages in the various multiples of dose represented in the ten dosages. (It can also be seen in the direct plotting of the data in Figure 2 in the discussion section.) The data seem to indicate that the rate-dose relationship is exponential for these classes of visible mutants.

To test for a possible exponential relationship the data were plotted on two-cycle double-log graph paper. Examination of the plot suggested that the relationship is not only exponential but two phased, with a change in phase occurring in the middle of the dosage range at about the 4 kr level. With this in mind a best-fitting sight line was drawn for each phase. The results are shown in Figure 1.

The lines in Figure 1 indicate that the mutation rate increased approximately as the 1.2 power of the dose in the 0.5 kr to 4 kr range and as the 1.5 power of the dose in the 5 kr to 10 kr range. The transition from one phase to the other may be more gradual than the lines suggest, and could conceivably be a continuous change from approximately linear at very low doses to the 1.5 exponential phase in the 5 kr to 10 kr range. For convenience it will be treated as essentially two phased as indicated by the lines.

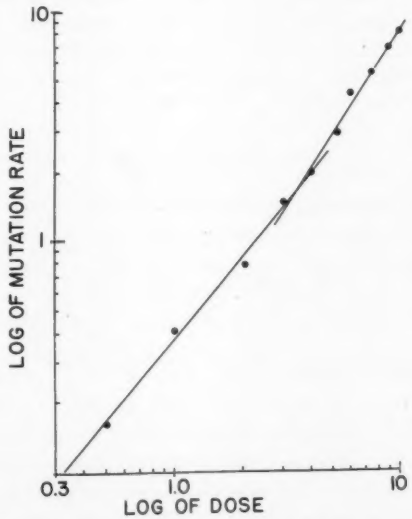

FIGURE 1.—The rate-dose relationship on a log scale. Vertical axis: percentile rate of visible mutations on a log scale. Horizontal axis: kr dose of γ radiation on a log scale. The points are the percentages given in the last column of Table 1. The lines were fitted by sight.

The data for Minutes and OB are not large enough to avoid the confusion of increased scatter when one plots them separately in a manner similar to Figure 1. But a glance at the individual percentile mutant rates at different dosage levels in Table 1 indicates that they are in general agreement in showing a faster rate of increase at doses above 5 kr than at doses below 4 kr. Thus the lines in Figure 1 can be reasonably interpreted as applying to both major classes of mutants. The *hes* data are too small to warrant such an analysis in detail, but at least they are not strikingly different from the other two classes in apparent rate-dose relationship.

Since there appears to be two phases to the rate-dose relationship it is of interest to compare the relative proportions of the different classes of mutants found in each phase range. The numbers of mutant flies are not large enough to warrant doing this on a dose-by-dose basis within each phase range. But they are large enough to compare the two ranges, group by group. This is done in Table 2.

The data of Table 2 show that the proportion of *hes* mutants was similar in the two dose ranges, and that Minutes were a little less frequent and OB a little more frequent, proportionately, in the 5 kr to 10 kr range. The change in proportion of Minutes and OB, though relatively small, is significant at the 0.1 percent level, indicating that the difference may be considered as established.

The relative proportions of the various *hes* phenotypes among the mutants may be compared in a similar manner. The data are given in Table 3. In this tabulation five cases of double phenotype, *th st*, were considered as five mutants of each single phenotype. Those two loci are closely linked and can be included in the same viable deficiency. But if one were testing the two loci separately, such a deficiency would appear as a mutant for whichever of the two markers one was using.

TABLE 2

Proportions of the major classes of mutations

kr range	Total mutants	Number and percent of major classes*					
		hes	%	*M*	%	OB	%
0.5 to 4	838	115	13.7	450	53.7	273	32.6
5 to 10	867	118	13.6	403	46.5	346	39.9
Total	1705	233	13.7	853	50.0	619	36.3

* For description of major classes see Table 1.

TABLE 3

Mutation frequencies at the hes *loci*

kr range	Number of mutations									
	h	*th*	(*th st*)	*st*	*p*	*cu*	*sr*	*e*	Total	Av.
0.5 to 4	7	7	(2)	17	20	7	31	28	117	16.7
5 to 10	6	14	(3)	19	18	18	30	16	121	17.3
Total	13	21	(5)	36	38	25	61	44	238	34.0

Since the total number of *hes* mutants observed in one dosage range is approximately the same as that observed in the other, one can compare the frequencies of the several loci directly, without recourse to percentages. Compared in this way, using the information in Table 3, the seven phenotypes can be seen to vary considerably in frequency. Considering the totals for the entire range of doses, there are too many *sr* and too few *h* mutants, each difference significant at the 0.1 percent level. Broken down into the two dose ranges, however, the numbers become too small to reveal clearly even two-fold differences in relative frequencies of mutants at the several loci. It can only be suggested that *th* and *cu* mutants seemed to be relatively more frequent and *e* mutants less frequent in the 5 kr to 10 kr range.

DISCUSSION

This study is concerned with the production of visible mutations by ionizing radiation in the mature sperm of *Drosophila melanogaster*. Each of the three classes of mutations observed here has long been known to be associated with various kinds of chromosomal mutations. (See MULLER 1954 for an extended discussion of the points which will be considered here and for earlier literature references.)

Mutations at the *hes* loci specifically have been studied by WARD and ALEXANDER (1957) who found them associated half of the time with cytologically demonstrable chromosome aberrations when occurring in flies treated with 3 kr of X-rays. Minutes, commonly associated with deficiencies and deletions when occurring after radiation treatment, have recently been studied by GLASS (1955) in a manner similar to the present study. Many of the visibles classed here as OB have frequently been found by others during the past 30 years as position effects or as mutations closely associated with chromosomal aberrations.

It is to be expected then that the rate-dosage relationship obtained in this study will be closely related to that established for chromosomal mutations. Mutational changes requiring only a single ionizing track (one hit) increase in rate in mature sperm of Drosophila normally as the first power of the dose, that is, linearly. Chromosomal rearrangements requiring two independent ionizing tracks (two hits) increase, under similar conditions, as the 1.5 power of the dose, the remainder of the amount of rearrangements represented in the 2.0 power (or square) of the dose being either lost or restituted.

In the present study, visible mutations increased in rate approximately as the 1.2 power of the dose in the 0.5 kr to 4 kr range of γ radiation and as the 1.5 power of the dose in the 5 kr to 10 kr range. Apparently there was a mixture of one-hit and two-hit mutants recovered after low doses (under 4 kr) and predominantly two-hit mutants after high doses (above 5 kr).

On this basis it appears likely that most of the visible mutants recovered in the 5 kr to 10 kr tests were associated with gross chromosomal rearrangements, probably as position effect mutants. Two direct observations in the present experiments support this interpretation. First, OB, the common kind of position effect

visible mutant, increased in rate a little more rapidly in the high dosage tests than did the rate of Minutes. Second, nearly all of the high dosage OB which were adequately tested showed the reduced crossing over which is characteristic of gross chromosomal rearrangements.

The question arises: would one expect enough recoverable chromosome aberrations to be produced in the 5 kr to 10 kr range to allow for so many position effect mutations? In concurrent tests of lethal X chromosomes recovered after 12.5 kr treatment (IVES 1959) it was found that some 40 percent of the lethal chromosomes were apparently associated with gross chromosomal rearrangements requiring two or more hits for their origin. Such chromosomal mutations should occur in all the chromosomes carried by radiated sperm. In the *hes* tests there was the chance to detect any of them irrespective of their specific chromosomal linkage, whenever a position effect visible mutation existed as a result of the aberration. A rough calculation on this basis indicates that recoverable chromosomal mutations probably outnumbered the observed visible mutants by a factor of at least two in the 5 kr to 10 kr range.

GLASS (1955) has also reported a two-phase rate-dose relationship in his study of Minutes, using X-rays and a more limited dose range. In his case the phase change occurred at a lower dosage level, about 2 kr, but at about the same mutation rate level, roughly one percent Minutes, allowing for small differences in the scoring of Minutes by different observers.

GLASS was able to continue scoring mutants in flies emerging well beyond the 18 day limit which was necessary in the present tests. He found a higher proportion of Minutes, particularly of extreme Minute phenotypes such as are normally associated with more extreme chromosomal changes, in late emerging flies. This could account for the difference in the dosage level of the phase change in these two independent studies.

The similarity of the Minute mutation rate level at which the phase change occurred in these two studies is especially interesting. It may be that, in a general way, for those combinations of irradiation doses and mutant eclosion time limits which together result in mutation rates of less than about one percent Minutes, the rate-dose relationship is linear or nearly so, while for combinations resulting in substantially more than one percent Minutes the rate increases as the 1.5 power of the dose, reflecting the predominating influence of two-hit rearrangements. This is a modification of GLASS's suggestion in connection with a difference between his results and those of IVES, LEVINE and YOST (1954). The latter study was not extensive enough at doses above 3 kr to test the present interpretation.

One of the major purposes of this report is to compare the results obtained in the study of mutants which are detected completely on the basis of phenotypic change with those obtained in the study of mutants which are detected completely on the basis of viability effects, in this case a lethal effect produced by the X chromosome as reported previously (IVES 1959). This comparison can best be made by the simple rate-dose plotting of the two sets of data which is given in

Figure 2. There is a marked contrast between the exponential relationship found for the visibles and the linear relationship found for the lethals.

It has already been indicated that the available evidence suggests that most of the visibles are probably associated with two-hit chromosomal aberrations, probably gross chromosomal rearrangements, in the 5 kr to 10 kr range. Some 60 percent of the lethal X chromosomes, however, appear to be free of gross chromosomal rearrangements. This in itself should decrease the influence of the rate of such chromosomal mutations on the rate-dose relationship for X chromosome lethal mutations as compared to its influence on the relationship for visibles. It may be expected, however, that some influence should be seen in the X-lethal rate-dose curve from the effects of the chromosome rearrangements which do occur, as represented in 40 percent of the 12.5 kr lethals.

There are at least five possibilities to be considered here. First, X-lethal mutations may possibly occur mostly as simple point mutations, independently of chromosome rearrangements. In such a case the resulting rate-dose relationship would be linear, as was actually observed in these experiments. Second, chromosomal rearrangements involving the X chromosome may very often maintain the

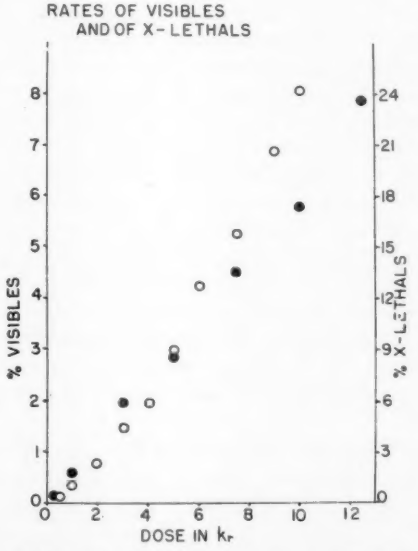

FIGURE 2.—Rates of visibles and of X-lethals in mature sperm of *Drosophila melanogaster* after cobalt-60 γ radiation. Open circles represent percentages of total visible mutations from Table 1, last column. Solid circles represent percentages of lethal X chromosomes similarly from Table 1 of IVES 1959. The percentage scales are adjusted to facilitate comparison of the two rate-dose relationships.

inheritance of position effect X-lethals which would be nonlethal in a normal gene sequence. If this were the predominant influence then the X-lethal rate-dose relationship should show exponential characteristics similar to those in the case of visibles, which it obviously does not. Third, if in their known destruction of cell lineages by abnormal division and separation, chromosomal rearrangements should also eliminate associated X-lethals which might be retained and recovered as single-break restitution lethals in lower dosage tests, then the X-lethal curve should increase less than linearly at doses above 5 kr, which it obviously does not do in the present instance. Fourth, if the effects mentioned as second and third here should both occur more or less equally, the net result could be a linear rate-dose relationship similar to what is actually observed. The present data can not be used critically in differentiating between the first and the fourth of these possibilities, in the opinion of the author. A fifth possibility, and the one which is most attractive to the author, is that all three types of effects occur (point mutation lethals, position effect lethals, and loss of potential restitution lethals) and that the net result happens to be approximately linear in the .3 kr to 12.5 kr range of Cobalt-60 γ radiation.

A final interesting point in the present observations concerns the chromosome region in which was located the loci of the most frequently occurring non-Minute mutations. The loci of the two most frequent *hes* mutants, *sr* and *e* alleles, and of the three most frequent OB, alleles of *Dl*, *H* and ss^a, are all linked together in a region of chromosome three which is only thirteen crossover units long. This region represents less than five percent of the total radiated euchromatin. It was the source of at least 20 percent of the OB, and its two *hes* loci produced mutations twice as frequently as did the five loci located elsewhere in this chromosome. While one is tempted to interpret this striking observation as indicating a chromosome region possessing exceptional sensitivity to γ radiation, several other possibilities can not be ignored, and there is no way to estimate their relative merits. First, these may be phenotypes to which the author is particularly sensitive as an observer. Second, this may be a region of the chromosome which is exceptionally well endowed, randomly, with genes which are more responsive phenotypically to position effects than is any similar number of genes in another comparably short chromosome region. Third, mutations induced by radiation in this group of genes may be less frequently dominant lethals than they are in other groups of genes.

The Stubble locus lies on the very edge of this unusual chromosome region. Among the many bristle mutations observed, none closely resembled Stubble, or second chromosome Bristle, and none when tested produced a population containing individuals resembling Stubble or Bristle. This suggests that probably not all the genes having the capacity for visible mutation and lying in this particular region happen to have the same responsiveness to the radiation treatment.

One can only conclude that this region of the third chromosome did contribute far more than its share to the observed non-Minute mutations. The reason for its doing so remains obscure at present.

SUMMARY AND CONCLUSIONS

1. The mature sperm of *Drosophila melanogaster* Oregon-R males were irradiated with Cobalt-60 γ rays and were tested for *hes*, Minutes (*M*) and other bilaterals (OB), the relative proportions being 14 percent *hes*, 50 percent *M* and 36 percent OB in the totals from ten dosage levels ranging from .5 kr to 10 kr.

2. Dominant mimic mutations of the *hes* loci are rare, and frequencies of the seven *hes* types are not equal, with too few *h* and too many *sr* (0.1 percent level of significance).

3. The total mutation rate increased as the 1.2 power of the dose between 0.5 kr and 4 kr and as the 1.5 power at higher doses, suggesting a mixture of one-hit and two-hit mutations at the lower doses and chiefly position effect rearrangements in the 5 kr to 10 kr range.

4. Both here and in GLASS (1955) the rate of Minutes shifted to the 1.5 power of the dose at about the one percent mutation rate point, but under different scoring conditions and at different dosage levels. It is suggested that the mutation rate rather than the radiation dose may be the important indicator of the change in phase.

5. The two-phase exponential rate-dose relationship observed here for visibles is compared to the linear relationship observed for X-lethals and is attributed in part to the apparently much lower proportion of chromosomal rearrangements in lethal X chromosomes and in part to a combination of other factors which are discussed.

6. The *ss–e* region of chromosome 3 contributed much more than its share to the total non-*M* visibles observed, a disproportion which may be due to any one or more of several indicated factors.

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